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# ANNUAL RESEARCH PROGRESS REPORT

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UNITED STATES ARMY INSTITUTE OF DENTAL RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, D.C., 20012

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Skin, Combat Casualties, Combat Wounds, Crown and Bridge Alloys, (cont. on reverse)

In-House Laboratory Independent Research - This program is instituted as one aspect of a broad approach to provide individual Army scientists and engineers an additional opportunity to maintain and increase their competence by doing original work in areas suiting their talents, thereby promoting a vigorous internal research program of the highest technical caliber.

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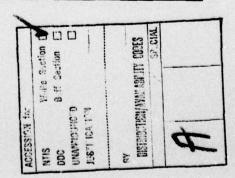
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US ARMY INSTITUTE OF DENTAL RESEARCH WALTER REED ARMY MEDICAL CENTER Washington, D.C. 20012

## ANNUAL RESEARCH PROGRESS REPORT 1 July 1976 - 30 September 1977

(The 7T period is included in this report)

DA Project	3A161101A91C	Task 00	In-House Laboratory Independent Research
DA Project	3S161102BS06	Task 04	Research in Biomedical Sciences - Dentistry
DA Project	3S762775A825	Task 00	Oral and Maxillofacial Sciences



#### FOREWORD

During FY 77 USAIDR research efforts have continued to develop, improve and bring to completion original initiatives for providing oral health care hardware, materials and methods in support of the combat soldier. Efforts also continue in the generation of baseline data for the continuing assessment of present and future obstacles to oral health care delivery.

In the area of hardware, two accomplishments are noteworthy. The testing of the USAIDR developed surgical scrub device (Hydroscrub) has been successfully completed and the instrument has been referred to USAMBRDL for type classification.

The prototype instrument developed by USAIDR for the more accurate determination of dental pulp vitality has been further improved and clinical testing continues to be promising.

In the area of materials and methods, there were several significant accomplishments in the development of implant materials. Methodology for forming PIA into artificial skin and hollow organ devices for a number of uses is well advanced and the feasibility of using these devices in treating avulsion of the esophagus and ureters has been established. Work on this important surgical modality continues. In addition the ability to fabricate degradable appliances as stimulators of circular and longitudinal fiber growth in hollow organs is a major step in hollow organ grafting. These tissue stimulators produce neogenesis of the anatomic morphology of recipient sites.

The use of degradable ceramic in obliterating cystic cavities in facial bone following trauma has given good results in experimental animals and its use in humans as a means of filling alveolar bone defects continues to show good results.

Studies on the occupational dust hazards encountered by dental personnel are serving a twofold purpose. First they are determining the potential for toxic sequelae from asbestos, metals and synthetic products used in the dental laboratory and clinic and more importantly these studies are providing a methodology base for use in the study of small particle effects from high-velocity missiles; part of a study just initiated.

The USAIDR has just undertaken two highly important cooperative studies with the Aberdeen Proving Grounds. The first, mentioned above, is a determination of the soft tissue and bone damage caused by the new high-speed missiles (5000 ft/sec and above). New hazards in the form of secondary missiles and dust sized particles will require the establishment of parameters for rapid and effective treatment.

The second study involves the ability to test rapidly, under combat conditions, for toxic substances used in the combat environment via saliva; a non-invasive approach. The long-range plan is to develop "chemical sticks" for determining various toxic substances.

## USAIDR PROJECTS, TASKS, AND WORK UNITS

(Responsible Division in Parentheses)

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DA OG 6038	The Viscous Properties of Endodontic Sealers (Dental Materials)	10

	the Maria (Santal Caterials).	ige No.
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DA OG 6041	A Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents (Oral Biology)	12
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DA OG 6039	Identification of Leukocyte Populations Responsible for the Production of Osteoclast Activating Factor and Their Role in Bone Resorption (Oral Biology)	18
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00	Oral and Maxillofacial Sciences	
DA OD 6048	Development and Evaluation of Nitinol for Use in Army Dentistry (Dental Materials)	19
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- with the polaroid pictures will be used to determine the effectiveness of the polaroids in providing an accurate treatment record.
- 25. (U) (76 07 77 09) Results of phase II of this study indicate that intraoral polaroid pictures are at least as accurate as panorex film in recording dental treatment. When compared against the written treatment record no significant difference was found between intraoral polaroid pictures and panorex film when used as a means of recording dental treatment. It is concluded on the basis of results from phase I and II of this study that intraoral polaroid pictures are of significant value in Army dentistry as a forensic tool, as a means of recording dental treatment, and as a means of decreasing exposure to x-irradiation.

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23. TECHNICAL OBJECTIVE, 24. APPROACH. 25. PROGRESS (Furnish Individual paragrapho Identified by number. Procedo lext of each with Socurity Closellication Code.)

- 23. (U) To design and fabricate prototype plastic rotary instruments to be used for debridement of avulsive type wounds, jaw fractures and extraction sites in the care of combat casualties. The design will allow removal of unwanted granulation tissue, necrotic tissue and cystic tissue without gross alteration of surrounding normal bone. It will therefore increase the speed and efficiency of surgical and periodontal procedures decrease healing time, and will constitute a cost effective procedure by returning soldiers to duty more rapidly.
- (U) Rotary instruments will be designed, fabricated and perfected at USAIDR.
- 25. (U) (76 07 77 09) Two designs have been proposed and made in pilot form. One design is intended to remove soft tissue adherent to bone without damage to the bone, the other, to remove free-standing, unsupported tissue. Both burs are made principally of Kel-F plastic, and have shown feasibility in laboratory tests. Both designs are intended to remove loose non-vital disorganized tissue, rather than cut, so that adjacent, vital tissues are left unaffected. Because minute amounts of the bur material could conceivably lodge in the tissue in use, it has been deemed advisable to investigate tissue compatibility of minute particles of Kel-F plastic. When suitability of this plastic, or another if necessary, has been confirmed, actual clinical testing will begin. Having established the feasibility of using a plastic surgical bur for debridement of avulsive type wounds, this work will be transferred to the 825 program.

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(U) Preventive Dentistry

23. (U) To determine if a dental flossing device (E-Z-denta-flosser<sup>R</sup>) has any advantages over hand-held floss that can be used in aggressive oral health program. It is imperative that more efficient means of teaching, controlling and preventing dental disease among soldiers be developed. A mechanical flossing aid which will improve the self-application of preventive dentistry measures among soldiers would result in reduced expenditures of funds required to treat dental diseases occurring among troops as well as reduce professional man-hours expended in treatment of troops.

- 24. (U) Military dental patients will use both hand-held floss and the flossing device for a period of two weeks. Controls will not use floss. Oral hygiene will be evaluated before and after the two week period in all patients using the interproximal sulcular gingival bleeding index. Patient preference for either method of flossing will be evaluated by questionnaire.
- 25. (U) (76 07 77 09) The patients who used flossing showed a significant improvement in oral health but no significant difference was seen between the two flossing groups who used either hand-held floss or the mechanical flossing device. However demonstration that the mechanical device was at least as effective as hand-held floss establishes the value of the mechanical device and permits its recommendation to patients who are either unable or unwilling to use hand-held floss. It is concluded that the mechanical flossing device used in this study is a valuable addition to military oral health programs.

\*Available to contractors upon originator's approval.

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22 KEY BORDS (Frecedo	elligence Cor	leation Code) ([1])	Sealants.	(11)	IV Light	: (U) O	cclusal R	estorations;	
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23. (U) To compare the treatment time required to restore fractured anterior teeth and the occlusal surfaces of permanent first molars using UV light cured filler and sealant with the treatment time required to perform the same procedures using conventional mehtods. While several reports have been published evaluating and recommending

the use of fillers and sealants, data on the time required for performing filler and sealant procedures are lacking. Before this preventive procedure can be recommended for adoption by the Army it must be compared for cost effectiveness against presently used methods.

24. (U) Military dentists will perform restoration procedures using both filler and sealants and conventional methods on a statistically valid number of patients. Treatment times will be recorded. All treatments will be reexamined one month following performance by an independent examiner to determine if restorations are satisfactory.

25. (U) (76 07 - 77 09) The average times required to restore fractured anterior teeth by conventional methods as compared to using a UV light cured sealant were 35.9 and 28.5 minutes respectively. The range of values obtained did not demonstrate any significant placement time difference between the two methods. A comparison of the times required to place an amalgam restoration vs. a sealant restoration in contralateral posterior teeth of 10 patients indicated that sealants required about half the time to place as amalgams but the failure rate after 30 days was 30% for sealants and 0% for amalgams. It is concluded that the time saved in using sealants is negated by their failure rate, particularly in the context of maintaining combat readiness.

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Sodium-Sulfonate; (U) Cysteamine-N-Acetic Acid; (U) Mercury Vapor

- 23. (U) To develop a drug of greater effectiveness, lower toxicity and broader application for the detoxification of mercury in military dental personnel. Presently available drugs are either effective only in acute toxicity situations or have toxic side effects. Since available data indicate that military dental personnel carry above normal levels of body mercury and the possible subclinical effects of metallic mercury are known it would be desirable to have a drug which could be routinely and safely administered for depleting above normal but not clinically toxic levels of mercury in dental personnel.
- 24. (U) Two promising drugs will be evaluated for their ability to deplete body mercury in small animals subjected to the inhalation of known amounts of mercury vapor. If effective these drugs will be evaluated for their toxicity and studies will be extended to primates.
- 25. (U) (76 07 77 09) Continued evaluation of 2,3 dimercaptopropane sodium sulfonate (DMPS) in experimental animals indicates that it is far superior to any drug in use or proposed for mercury detoxification. A three day course of 30 mg/kg/day of DMPS removed up to 76% of body mercury in rats. Comparison with N-acetyl penecillamine at present the most effective experimental mercury detoxifying agent, reveals that DMPS is at least 8 times more effective. No apparent toxic effects have been noted thus far with DMPS administration to small animals. The data suggests that DMPS may be an ideal drug for use in the prevention of body mercury accumulation. The work is being transferred to the BS06 program.

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NAME: Cutright, D.E. COL, DC				TELEPHONE: 202-576-3258							
TELEPHONE:	202-376-3484			SOCIAL SECURITY ACCOUNT NUMBER:							
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(U) Dental History; (U) Medical History; (U) Dental Therapy; (U) Patient Ouestionnaires

- 23. (U) To determine the adequacy and accuracy of individual medical histories obtained in Army dental clinics by comparing them with the patients medical record. The current practice in dental clinics of obtaining medical histories by patient question-naires could lead to therapeutic error, duplication of effort, delayed treatment, increased costs and increased professional man-hours in patient care. The results of this study will point to any needed improvements in the present system.
- 24. (U) Six hundred randomly selected Army dental records will be examined. Information from the corresponding medical history will be compared with the medical information on the dental record. Discrepancies will be tabulated and analyzed.
- 25. (U) (76 07 77 09) A discrepancy rate of 39% was found between the medical histories of patients as recorded in their dental history and their original medical history. Eleven percent of the patients were judged to have significant omissions of medical conditions and/or treatment, e.g. active treatment for infectious disease (5%); unspecified drug allergies; or on-going medication with drugs having potential dental therapeutic contraindications (6%). It is recommended that (1) the patients medical record be reviewed at the initial oral exam (2) there be a thorough verbal follow-up to the written health questionnaire and (3) a recheck be done before prescribing medications.

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(U) Wound Sterilization; (U) Wound Debridement

- 23. (U) To evaluate the CO<sub>2</sub> surgical laser for use in the management of oral-facial combat wounds. Available data indicate that 10-12% of combat wounds and 7% of non-combat wounds requiring hospital care involve the maxillofacial region. The successful application of the CO<sub>2</sub> surgical laser to microsurgery in the aerodigestive system has demonstrated a number of highly desirable characteristics relative to maxillofacial surgery. These include hemostasis, no postoperative edema, minimal postoperative scarring, sterilization of infected wounds, minimal pain and a sharply demarcated operative field. This suggests that the CO<sub>2</sub> laser may provide a rapid, safe and superior approach to the debridement and subsequent reconstruction of maxillofacial wounds. A more effective modality of managing maxillofacial wounds would result in significant savings in hospital costs and professional man-hours and effect a rapid return of the soldier to duty.
- 24. (U) A CO2 surgical laser will be utilized to establish baseline reactions in normal oral tissues of experimental animals. Subsequently simulated maxillofacial wounds (to include teeth, bone and soft tissue) will be treated to evaluate the feasibility of using the surgical laser for hemostasis, incision, excision and debridement. The rapid sterilization of selected instruments and appliances will also be studied.
- 25. (U) (76 07 77 09) The surgical laser was received June 77. Preliminary studies on the oral mucosa of rhesus monkeys indicate that the surgical laser produces predictable tissue effects with ease, precision, speed and lack of hemorrhage. Sterilization of metal appliances, endodontic instruments and periodontal scalers contaminated accidentally during surgery was found to be feasible in as little as 30 seconds without damaging appliance geometry or instrument cutting edges.

  \*Assistate to Controllers upon originators approved.

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RESPONSIBLE INDIVIOUAL  NAME: Cutright, D.E., COL, DC  TELEPHONE: 202-576-3484  21. GENERAL USE  Foreign Intelligence Considered				PRINCIPAL INVESTIGATOR (Purels SEAN II U.S. Academic Invitation)  NAME: Gross, A., COL, DC  TELEPHONE: 202-576-3764  SOCIAL SECURITY ACCOUNT NUMBER:  ASSOCIATE INVESTIGATORS  NAME: Setterstrom, J., PhD  NAME:						

- (U) Liquid Chromatography; (U) Lipids
  2) TECHNICAL OBJECTIVE. 24 APPROACH, 25 PROGRESS (Furnish Individual paragraphs Identified by number. Proceeds load of each with Security Classification Code.)
- 23. (U) To develop an improved, rapid, sensitive and precise method for the identification of Pathogenic bacteria associated with combat wounds. A critical phase in the successful treatment of combat wounds is the early detection and identification of potentially destructive pathogenic organisms and the institution of appropriate therapy. Instrumental techniques are now available which may make it possible to identify unique constituents from different microbial genera thus offering the possibility of rapid and accurate identification of pathogenic organisms directly from body fluids and wounds. Such a procedure could eliminate lengthy and sometimes difficult culturing procedures, reduce man-hours expended in the laboratory, reduce professional man-hours in patient treatment, speed wound healing, lower morbidity and be a positive moral factor for the combat soldier.
- 24. (U) Currently used gas chromatographic methods as well as highly sensitive liquid chromatography methodology recently developed at USAIDR, will be used to identify cellular fatty acids and metabolic by-products by wound infecting microorganism.
- 24. (U) (76 07 77 09) Studies to date indicate that the best results are obtained by high-performance liquid chromatography (HPLC). Sufficient sensitivity, separation and working range has been obtained in identifying and quantitating cellular fatty acids by HPLC to conclude that the present approach shows great promise as a rapid accurate means of identifying wound infecting organisms. The detection of vaccenic acid (< 0.1%) in oral streptococci is an example of the sensitivity and separation obtainable with HPLC which has eluded other powerful methodologies.

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13. TECHNICAL OBJECTIVE. A APPROACH. 23. PROGRESS (Furnish Individual paragraphs Identified by number. Procedu test of each with Security Classification Code.)

- 23. (U) To increase the effectiveness of electroless plating as a means of preventing pit and fissure caries. Current research has indicated the feasibility of using electroless plating for this purpose. Present systems however are limited by problems in the solubility and stability of the metal salts which make up these systems. Chelating agents may offer an approach to solving these problems and the possibility of developing new and improved electroless plating systems.
- 24. (U) Presently developed electroless plating systems will be prepared with and without various concentrations of different chelating agents and the resulting preparations will be compared for stability and effectiveness in forming an adherent seal on the tooth surface. Attempts will be made to solubilize and stabilize new heterogenous systems of metals using chelating agents and to effectively plate these systems on teeth.
- 25. (U) (76 07 77 09) A total of four chelating agents have been evaluated for their ability to stabilize AgNO3 and AgF solutions used in the electroless plating process; cysteamine-N-acetic acid, N-acetyl penecillamine, cysteamine and sodium-2, 3-dimercaptopropane-1-sulfonate. Both AgNO3 and AgF solutions in water were stabilized for periods up to 3 weeks in the absence of light. While none of the chelating agents interfered with the electroless plating process no improvement of the resultant plate was observed. The overall results obtained did not warrant the addition of chelating agents to the electroless plating system studied.

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- 23. TECHNICAL OBJECTIVE. \* 24. APPROACH, 25. PROGRESS (Pumish Individual pergraphs identified by number. Precede text of each with Security Closelfication Code.)
- 23. (U) To determine the rheological properties of endodontic cements of interest to the Army. The data will (1) establish the definitive criteria for selection of materials suitable for military use and (2) Establish techniques relevant to manipulation and clinical use of viscous cements. The information obtained will significantly enhance the ability of the Army dentist to render endodontic treatment and thus result in savings of fiscal and professional manpower resources.
- 24. (U) Viscous properties of 7 root canal sealers will be characterized by capillary extrusion and rotational viscometry. Effects of time, method of measurement and shear rate will be evaluated. Techniques for effective clinical placement of endodontic sealers will be refined and developed.
- 25. (U) (76 07 77 09) Viscosity tests performed on 7 root canal sealers indicated wide variations in initial viscosity with marked differences in the rates of increase in viscosity with time. At least four discrete patterns of flow behavior were noted involving low or high initial viscosities and either the absence of change or marked increases in viscosity with time. The study has made possible the recommendation of specific materials to meet the clinical needs of both the experienced and inexperienced operator and provides the background required to further improve endodontic sealers of interest to the military dentist.

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- (U) Individual Identification; (U) Combat Casualties; (U) Mass Casualties
- 23. (U) There is no personal identification system available which allows identification of living or dead individuals under conditions of fire, drowning, combat and other casualty situations. This method will utilize a non soft tissue invasive method. If proven, it will allow identification of the living, dead or even ashed remains under certain circumstances.
- 24. (U) A metal pin of a noncorrosive metal with a high melting point will be used (medical wide stainless steel). This metal will be fabricated into a very small "rivet" shaped pin of approximately  $2\frac{1}{2} \times 1\frac{1}{2} \text{ mm}$ . This will be placed in teeth with appropriate identifying numbers on its shaft and head. Feasibility studies will be with extracted human teeth.
- 25. (U) (76 07 77 09) The device has been constructed, the sample identifying numbers inscribed on both the head and shaft and the device placed in several teeth using a specially altered dental bur. The feasibility and practicality of its use for identifying dead or unknown unconscious individuals has been shown. A patent disclosure has been filed.

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In Title (Precede with Security Classification Code)* (U) A Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents											
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(U) Salivary Protein; (U) Salivary Electrolytes

- 23. (U) To determine if saliva can be used as a diagnostic tool in evaluating the exposure of combat troops to lethal agents. To determine if parameters in saliva can be used to monitor the progress of therapy for lethal agent exposure. Develop a rapid simplified field technique for identification of lethal agent exposure in the combat soldier.
- 24. (U) Changes in saliva produced by lethal agent exposure will be evaluated. The particular areas of study will be protein, electrolyte and immunological components. Possible methodology developed will be evaluated in the field and at the hospital level.
- 25. (U) (76 07 77 09) A saliva collection technique has been successfully initiated in rhesus monkeys and is currently being optimized. Saliva collected to date is being studied by gradient gel electrophoresis, a new and highly sensitive technique. The accumulation of baseline data for the normal monkey is nearing completion. Additional monkeys are on order for continued testing.

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(U) Electric Current (U) Prostaglandin E, (U) Cyclic AMP, (U) Phosphodiesterase 3. TECHNICAL OBJECTIVE. 24 APPROACH, 28. PROGRESS (PM

- 23. (U) Studies conducted by the Army in recent years show that 10-12% of combat wounds involve the maxillofacial apparatus. Further 7% of noncombat injuries requiring hospital care involve the maxillofacial region. This results in the loss of approximately 1,000,000 man-hours per year. The research objective is to accelerate or otherwise improve healing of the combat maxillofacial wound, demonstrate cost effective measures by decreasing medical requirements and hospital stay and effect a rapid return of the soldier to duty.
- 24. (U) Studies on the effects of biochemical and physical factors to include chelate complexes, cyclic AMP, prostaglandins, scar inhibiting agents, in vivo growth factors and electric currents on the rate of healing in soft tissue and bone will be done. The mechanism of any beneficial alteration in healing effected will be investigated and pursued to human usage.
- 25. (U) (76 07-77 10) The effect of PLA films containing cAMP on oral wound healing was studied in rhesus monkeys. The data are being analyzed. Studies continue on the effect of anti-phosphodiesterase agents on inflammation and healing in oral wounds. A study of the effects of time of surgery on prostaglandin E, phosphodiesterase and cAMP showed that only cAMP levels change with increasing time of surgery. Continued study of the effects of small electric currents on bone healing have not shown consistent results. Constant current levels between 2 and 10 µa increased bone deposition above conrol levels in mandibular injuries in 7 of 9 animals. Constant currents of 25 and 50  $\mu a$ gave negative results.

#### ACCELERATION OF WOUND HEALING

#### THE EFFECT OF CAMP AND PLA ON WOUND HEALING

Wounds healing is a complex process in which epithelial as well as fibroblastic repair are occuring at the same time. The object of this study was to see if the rate of collagen systhesis in the wound could be stimulated while retarding the rate of epithelial growth. Lactic acid and cAMP have both been shown to stimulate fibroblast growth in vitro while cAMP has been shown to stimulate fibroblast growth in vitro and in vivo and appears to regulate epithelial growth. It was therefore decided to see what effects PLA alone, and PLA containing cAMP had on wound healing. If wound healing can be regulated in a predictable manner then treatment of combat wounds could be done in a more efficient and scientific manner.

The effects that dressing of PLA and PLA containing cAMP have on wound healing was studied in 8 rhesus monkeys. The model system used to study oral wound healing was gingivectomy done on the facial aspect of the maxilla and mandible from the lateral incisors to the 2nd molar.

The gingivectomies done on each animal were done in equal quadrants. two control areas dressed with coe pac, one dressed with PIA alone and one dressed with PIA - cAMP. The plain PIA dressing was derived from a lyophylized methylene chloride solution of PIA. The PIA - cAMP combination was similarly prepared from a mixture of a methylene chloride - PIA solution with an absolute ethanol solution of cAMP. Tissue samples for biochemical analysis and histological evaluation were taken at zero time, 7 days and 14 days.

The collagen content, phosphodiesterase activity, protein content, cAMP content and cAMP binding activity of the tissues have been determined and the data are now being statistically analyzed. Histologic analysis has not yet

been reported. Preliminary results do not show any effects on phosphodiesterase activity. In the future the method of polymer film production will be modified so as to produce a thinner and more flexible film. The polymer film used in this experiment was somewhat brittle and tended to produce some tissue irritation due to its mechanical properties. We are now able to produce soft cotton-like polymer complexes or pliable films which stick to exposed tissue surfaces.

## EFFECT OF ANTI-INFLAMMATORY AND ANTI-PHOSPHODIESTERASE AGENTS ON INFLAMMATION AND HEALING OF GINGIVA

Attempts were made to assay the adenylcyclase activity present in monkey gingiva to determine the effects that local agents or trauma would have on its activity. The amount of tissue removed was not sufficient to enable us to measure the activity of this enzyme. Therefore future studies will have to use larger samples or a more sensitive assay.

#### EFFECTS OF SURGERY ON GINGIVAL PROSTAGLANDIN E CAMP AND PHOSPHODIESTERASE LEVELS

Prostaglandin E (PGE), cAMP and cyclic nucleotide phosphodiesterase have been implicated in tissue inflammation, epithelial growth and fibroblast metabolism. The effects of prolonged surgery and trauma on the levels of these compounds was studied to determine if changes in their levels might be related to wound complications frequently seen in later phases of healing. The surgical model consisted of full thickness facial gingival flaps, extending from canine to canine, on the maxilla and mandible of 9 rhesus monkeys. Gingivae on the right sides were removed at the time of initial surgery and served as the controls. The flaps on the left sides served as the experimental areas and were reflected for 30, 60 or 120 min. (3 monkeys at each time). The basal level of PGE in maxillary gingiva, determined by radio-

immunoassay, was 182.1±47.8 picograms/mg protein (N=8). The phosphodiesterases in gingival homogenates hydrolyzed 14.0±1.9 picomoles of (<sup>3</sup>H) cAMP/mg protein/10 min (N=8). The levels of PGE and phosphodiesterase in the gingival flaps did not show any significant changes after surgery. The basal level of cAMP in the mandibular gingiva, determined by radioimmunoassay was 6.1±0.8 picomoles/mg protein (N=9). The levels of cAMP in the experimental showed a maximal 75% reduction (p<0.025) at 120 min after flap reflection. These reductions did not appear to be due to tissue changes in PGE content or phosphodiesterase activity. The decreased levels of cAMP during prolonged surgery may be one of the factors responsible for the incidence and degree of complications seen in later phases of healing combat wounds.

#### THE EFFECT OF ELECTRIC CURRENT ON BONE HEALING

In previous work we have found that direct electric currents in the range 2 to 10 microamperes stimulated bone healing but gave inconsistent results. Difficulties were experienced with large variations in current flow and destructive anodic effects.

Studies during the past year have been directed toward a redesign of the current delivery system in our guinea pig model, a study of the effect of constant direct currents in the range 2 to 50 ma on the healing of mandibular bone injuries and preliminary design studies in applying constant electric current to bone healing in primates.

The redesign of the current delivery system in our guinea pig model was dictated by frequent failures of the system resulting from intervention by the experimental animal as well as current fluctuations of unknown origin and destructive anodic effects. A constant current system using a field effect transistor was designed for stationary attachment to the animal cage and connection to the animal through a flexible stainless steel cable free

to swivel with the movements of the animal. The cable was attached to the animal via a bracket surgically implanted on the skull and platinum electrode wires were run subcutaneously to the injury site. Injuries consisted of the removal of 2 mm diameter bone plugs from either side of the inferior aspect of the guinea pig mandible. One injury site received an active platinum cathode and the other a dummy cathode. The anode was implanted beneath the skin on the superior aspect of the neck.

Three animals were studied at each of 5 current levels; 2, 5, 10, 25 and 50 microamperes. In 7 of the 9 animals treated in the range 2 to 10 ma, some improved bone healing was observed in the experimental injury site as compared to the control injury site. However the results varied considerably. Although current fluctuations and destructive anodic effects were controlled the same inconsistencies were seen as noted in previous work. At current levels of 25 and 50 ma little or no improvement in bone healing was seen. This finding is not in agreement with studies reported in the literature which indicates that currents up to 100 ma produce positive healing effects.

Preliminary design has been completed of a current delivery system for use in primates which is similar to that used in the guinea pig. The retirement of one of the co-investigators in this study has prevented its advancement to primates during FY77 as previously planned.

#### THE EFFECT OF PLA AND CERAMIC ON BONE & SOFT TISSUE HEALING

Sixteen guinea pigs were used to test the effects of 100% tricalcium phosphate and a mixture of 50% tricalcium phosphate and 50% biodegradable polymer mesh on bone healing. Three mm OD defects were placed in the right and left tibias of each guinea pig. The right defect was filled with the polymer ceramic mixture while the left defect was filled only with ceramic. Four guinea pigs were sacrificed at 7, 14, 21, 28 days post surgery.

The healing response was evaluated by measuring the collagen, protein and acid phosphatase activity of the implants. Biochemical analysis of this phase has been completed and statistical analysis is under way. The magnesium and phosphorus content of the defects remain to be determined.

In addition the soft tissue response to PLA mesh was evaluated by placing implants to 100% PLA foam in the abdominal area of each of the 16 guinea pigs. The implants removed at 7, 14, 21 and 28 days were histologically evaluated and the collagen content as well as acid phosphatase activity determined.

Histologic analysis showed that the PIA implants were accepted by the tissues and showed ingrowth of collagen tissue. However, at 28 days little degradation of the implant had occurred. It thus appears that the maximal ingrowth of collagen into the implant occurred at 21 days and at 28 days some resorption or remodeling of the collagen was occurring. This reduction in collagen content may result from breakdown of the implant which may have begun at this time. The alkaline phosphatase activity in the implant showed a fairly constant activity except that at 28 days it dramatically increased. The maximal acid phosphatase activity was noted at 14-21 days post surgery and showed a decline at 28 days post surgery. The changes seen in these enzyme activities may play a role in the collagen ingrowth and breakdown of the implants by the body. If experiments continue to show positive results the possibility of altering the healing rate of wounds may be possible.

# EFFECTS OF CERAMIC AND PLA/PGA MESH FILLED WITH CERAMIC POWDER ON BONE HEALING AND PH IN DEFECTS IN RAT CALVARIA

This experiment was done using 96 rats. Forty-eight rats had 3 mm OD defects made in the right and left calvaria. The defect on the right side was filled with a mixture of 1g ceramic + 1.64 g 50-50 PLA/PGA powder, while the left side was unfilled. In the second group of 48 rats the defect

on the right side was filled with only biodegradable ceramic and the left defect was left unfilled. Samples were taken at 3, 7, 14, and 18 days post-operatively.

The rationale behind this experiment was to see if those compounds tested could form bone in an area which normally does not heal by bone fill-in. This is of paramount importance in the treatment of combat causes skull injuries.

The following parameters were assayed: wet weight, dry weight, pH, protein content, acid and alkaline phosphatase activity, hydroxyproline content, histology and cAMP content.

Stastical analysis of the results is in progress. The following tentative conclusions can be drawn:

- 1. Histologically, no new bone formation was noted up to 28 days.

  However, the ceramic and the PLA/PGA ceramic filled defects both stimulated a greater amount of fibrous tissue growth to fill in the defects than was noted in the control defects.
- 2. The pH of all the defects was similar to the control defects, that is, neither the ceramic filled nor ceramic-polymer filled defects showed any significant differences. The pH of the original bone plugs and the repair tissue was between pH 7.0 and 7.5.

The mineral analysis of the repair tissues still has to be done.

A new experiment in which the longterm effects of ceramic polymer mixtures on bone formation in skull defects is being planned.

#### **PUBLICATIONS**

Levin, M.P., Grower, M.F., Cutright, D.E. and Getter, L.: Effects of Length of Surgery on the Healing of Full and Partial Thickness Flaps. J. Oral Path. 6: 152-160, 1977.

Grower, M.F., Chandler, D., Kramer, G.: Regulation of Gingival cAMP Levels Effects of Surgery, Lidocaine and Epinephrine. J. Dent. Res. 56 (Special Issue A) 1977.

Grower, M.F., Chandler, D., Kramer, G, and Murphy, D.: Effects of Surgery on Gingival Prostaglandin E, cAMP, and Phosphodiesterase Levels. J. Dent. Res. 56 (Special Issue B) 1977.

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- 13. TECHNICAL OBJECTIVE, 24. APPROACH, 28. PROGRESS (Pumlish Individual paragraphic Identified by number. Procede text of each with Security Classification Code.)
- 23. (U) To evaluate the special military problems of drug storage, heat susceptibility, long-term drug potency, sterility of bulk items, lack of refrigeration in combat zones and delivery to the patient. To investigate drug hazards. To investigate the use of biodegradable polymers for the long term, slow release delivery of drugs.
- 24. (U) Experiments will be conducted to evaluate pain killing medicaments used in military dental practice. The hazards involved in the use of various drugs will be studied and improved means of drug delivery will be investigated.
- 25. (U) (76 07 77 10) Thin films and hollow tubes to be tested as artificial skin and hollow organ grafts respectively have been developed from polylactic acid solutions A PIA-eugenol mixture with potential as a temporary filling material has been prepared. Studies on the use of PIA/PGA-medicament combinations for in-vivo slow drug release show promise. Data indicating that the eugenol used in dentistry may have a limited shelf-life has been obtained. A number of dental products have been found to be potential inhalation hazards to dental personnel and patients. Continued studies on mercury levels in dental personnel and facilities indicate mercury hygiene problems in some clinics. Continued study of Sodium 2,3 Dimercaptopropane sulfonate (DMPS) as a metal detoxicant indicates that, unlike BAL (used for the same purpose) it can be given orally in a crystalline form which has a long term shelf-life and it may be useful in multielement detoxification. DMPS is also more effective and safer than BAL.

# THE PROBLEMS INVOLVED IN MILITARY ORAL HEALTH CARE DELIVERY RELATED TO THERAPEUTIC AGENTS & MATERIALS

## FABRICATION OF ARTIFICIAL SKIN AND HOLLOW ORGANS USING BIODEGRADABLE POLYMERS OF PLA-PGA

This study has had at its aim the fabrication of biodegradable polymers as drug delivery agents and as organ replacements. At the present time there is no commercial substitute available for skin or tissues to use in this type of grafting. If such material were developed it would simplify treatment of wounds, decrease hospital care, speed return to duty and reduce the expense of treatment. The following properties of PIA polymer solutions have been determined.

- 1. The technology of fabricating copolymers into fibrils and forming into different tissue simulating shapes has been extremely difficult and costly. Woven or spun appliances made of copolymers have been very expensive. The USAIDR has developed a method of spraying a degradable tissue simulating copolymer which gives fiber orientation in any desired direction. Hollow organs are given both a longitudinal and circumferential orientation. This allows the neogenesis of fibrous connective tissue in the same orientation and gives good physiological morphology and strength.
- lml of water can be combined with 10ml of 100% PLA or 50-50
   PLA-PGA (4 gm/polymer/50ml of methylene chloride) by sonicating the solution.
- The optimal polymer concentration for fabrication of spun polymers is 4 gm/PLA per 50 ml methylene chloride.
- 4. Hollow polymer tubes 2, 3, 4, and 5mm in ID have been prepared for use as substitutes from blood vessels, esophagus, and urethra. The esophageal implants have been implanted in rats with a survival time of up to 2½ weeks. The histologic response is being evaluated. Implants have also been used to replace dog urethra. The animals have not yet been sacrificed.

- 5. PLA polymer films have been prepared with a mesh-like composition by spraying the polymer on glass. These films are now being evaluated for use as an artificial skin. The response and toxicity of PLA (4gm PLA/50 mg of methylene chloride) sprayed directly on open wounds is also being evaluated.
- 6. A mixture of PIA in eugenol for use as a temporary filling or filling base has also been prepared. The addition of PIA to the eugenol appears to make the ZnOE thicker than plain ZnOE. The testing of the physical properties as well as the biologic acceptability of this compound remains to be done.

# PREPARATION AND CHARACTERIZATION OF PLA/PGA MEDICAMENT COMBINATIONS FOR USE IN COMTROLLED RELEASE OF BIOLOGICALLY ACTIVE AGENTS

During the last year work has continued on the preparation of copolymers containing medicaments (specifically actinomycin D). Previous
studies had indicated that the fastest way of dissolving the 50/50 PIA/PGA
polymer was to use a solution of 4% Hexaflouroisopropanol (HFIP) in methylene
chloride. However, after several experiments it was determined that the
HFIP reacted with the Actinomycin D and as a result the polymers prepared
by this method were of little value. It was concluded from these experiments that HFIP should not be used for incorporating medicaments into copolymers and that methylene chloride alone should be used for this purpose.

During the course of our work we developed a method of analysis for the quantitation of Actinomycin D in polymers both before and after implantation. This was done using a high performance liquid chromatograph equipped with a UV-visible detector and monitoring at a wavelength of 436nm. The separation was accomplished on either a  $\mu$  bondapak CN column using 55-60%

Methanol/H<sub>2</sub>O eluant or a fatty acid column using 75-80% Mehtanol/H<sub>2</sub>O eluant. This method enabled us to quantitate the amount of Actinomycin D but did not indicate its biological activity. Current studies are in progress using different medicaments which will permit determination of both the concentration of the drug and its biological activity. This should give us a better evaluation of the release of various active medicaments from implanted copolymers.

# PREPARATION, PURIFICATION AND USE OF EUGENOL FOR ZINC OXIDE/EUGENOL CEMENTS

Zinc oxide/eugenol cements are widely used in dentistry as temporary filling materials, cavity liners for pulp protection, capping materials, temporary cements in fixed prosthesis, impression materials and major ingredients of endodontic sealers. Recent studies have shown zinc oxide/eugenol to be highly irritating to the tissues, causing necrosis of the bone and cementum. Previous work on the purity of eugenol indicated as many as twenty-five different components in a single eugenol sample. Recent work on USP Grade eugenol (purity >95%) has demonstrated that different brands contain different impurities.

A method for the purification of eugenol was developed using normal phase liquid chromatography. This micromethod was then scaled up to a preparative liquid chromatograph and 30ml of eugenol was purified. Over 95% of the impurities previously seen were removed and as a result the eugenol had a purity of greater than 99.75%. Subsequent analysis (four months later) of the purified sample indicated that the eugenol did break down with time but not to the extent of the original sample. This could possibly indicate the need for expiration dates being placed on eugenol bottles. Use of the fresh

purified eugenol in animal experiments is in progress. This finding indicates that under military purchasing and storage conditions an expiration date should be utilized.

## THE EFFECT OF TOPICAL ANTIHISTAMINE ON THE INITIAL PULPAL IMPLAMMATORY RESPONSE OF MONKEY TEETH

One of the largest causes of loss of duty time in Vietnam and in CONUS maneuvers was pain of dental origin; a frequent contributor to this type of pain is inflammation of the dental pulp. This study was done in order to investigate the efficacy of using topical antihistamine therapy to reduce inflammatory changes associated with pulpal irritation, decrease time lost due to emergencies and effect a rapid return to duty.

Cavity preparations were made in sixty sound teeth of healthy monkeys. Thermal trauma was delivered to the pulps of all teeth through application of a heated calibrated soldering iron to the floor of the cavity preparations. Fifteen teeth were randomly designated as control and were restored immediately with zinc oxide-eugenol. Forty-five teeth were designated as test teeth and treated topically with a four percent antihistamine solution, and restored with zinc oxide-eugenol. Histologic sections were prepared to show the effect of the antihistamine on the thermally initiated inflammatory response during the post operative period.

No significant difference was observed in the inflammatory reaction of treated or untreated pulps. A four percent aqueous solution of topically applied antihistamine did not appear to be of any significant value in obviating pulpal inflammation.

# SEM AND X-RAY MICROPROBE STUDY OF POTENTIALLY TOXIC DUSTS IN DENTAL PRACTICE

Military dentists and military dental patients are exposed to a greater number of airborne hazardous substances, due to the military type practice. Included are submicroscopic particles which result principally from the use of the highspeed air turbine dental handpiece. Hazards due to submicroscopic airborne particles have yet to be examined and are or particular concern to the military, because it values long-term service from the career dentist and the career soldier; both groups being subject to 20-30 years of exposure to these substances if no control is applied.

In this experiment the SEM and x-ray microprobe was used to characterize the usual submicroscopic airborne particles encountered in daily dental practice. Parameters studied were size, shape, and elemental composition. These data will be used to identify particles having the greatest toxic potential due to size, shape and composition, as well as serve as a reference for identification of the particles in lung tissue and oral tissue during biopsy study either in routine pathologic examination or of experimental materials.

Substances studied were jet acrylic, tray acrylic, zinc cement, ceramco-metal and porcelain, jeltrate alginate, pumice, stone, and zircate material. These materials ere examined under actual conditions of use; dust, if generated, was collected at 10-16 cm from the source. Particles if generated in the mouth were collected 3 cm from the source. Specimens were collected on double-sided tape, carbon-coated, and examined in the SEM and microprobe.

Particle characteristics as to size, shape, and x-ray emission spectra were recorded. Spectra shape records will be used to determine the identity

of the substances studied in future biopsy material. Size characteristics indicate particular hazards in the 1-10 µm range, with special hazard in the case of the sharp edge shapes. It is concluded that special care must be exercised in the control of dust containing jet acrylic, porcelain (ceramco), alginate, and stone, particles ranging in size from 1-10 µm. The inhalation hazards exist for the entire dental staff and patient. Further study is necessary to evaluate hazards involved with large, angular, narrow particles of ceramco, porcelain, metal present in the oral cavity after intraoral grinding of this material.

## MERCURY LEVELS IN ARMY DENTAL PERSONNEL AND FACILITIES

A total of 116 blood and urine mercury determinations done to date on Army dental personnel indicate a positive correlation between the mercury levles found and the volume of restorative work done in a given clinic. This poses a particular military risk due to the large numbers of dentists operating in a single clinic.

The type of clinical facility also appears to be a factor in the level of mercury hygiene problems. Of 3 clinics at one post, a converted barracks-type clinic had the highest level of atmospheric mercury (0.04 mg/cu meter). The other 2 clinics designed specifically as dental clinics gave atmospheric mercury values of less than 0.01 mg/cu meter. All but one of the individuals displaying above normal blood (> 10 ng/ml) and/or urine (> 20 µg/l) mercury levels worked in the barracks-type clinic and represented 10 of the 18 individuals in that clinic. Of the remaining 8 individuals 5 displayed urine mercury values between 15 and 20 µg/l. None of the above normal blood and urine mercury values were indicative of mercury toxicity but they did indicate poor mercury hygiene.

Some evidence of mercury spills was found in all clinics evaluated.

Mercury contamination was found most often around amalgamators and in cabinets housing these devices. The highest mercury value found was 60 µg/liter in the urine of an enlisted man who had cleaned up a recent mercury spill.

A salient finding of this investigation to date has been the lack in Army dental clinics of adequate means of handling relatively large accidental mercury spills. A method involving the modification of equipment already available in Army dental clinics is currently being investigated in order to provide greater safety in policing mercury spills.

Work is continuing. Additional data will be followed closely for indications of problem areas and possible solutions.

### SODIUM 2,3 DIMERCAPTOPROPANE SULFONATE (DMPS) IN THE TREATMENT OF MERCURY POISONING

Studies on the use of DMPS in the treatment of metal poisoning have continued. A determination of the effect of DMPS on the excretion of essential trace metals during use in the treatment of mercury poisoning in experimental animals indicates that copper is excreted in moderate levels while zinc and manganese levels are unaffected. The level of copper excretion is such that prolonged chelation therapy with DMPS could require copper supplementation or attention to the intake of copper containing nutrients. The data also indicate that DMPS can be used as a useful detoxifying agent for copper and possibly chromium, nickel, bismuth and cadmium. Arsenic is removed from experimental animals with an effectiveness equal to that of mercury removal. The use of DMPS in multielement detoxification such as may be encountered in combat is being studied.

Unlike BAL, DMPS can be administered orally for the treatment of mercury intoxication. Oral DMPS is equally as effective in experimental animals as injected DMPS when given at approximately twice the dose level of the latter. DMPS retains its potency in crystalline form in sealed containers for at least one year at temperatures ranging up to 30°C. Thus far the data indicate that DMPS represents a significant improvement over BAL in the treatment of selected metal intoxicants and is particularly useful under field conditions where maximal safety, minimal professional supervision and simplified delivery are critical factors. Emphasis is being placed on the development of a detoxicant that can be used by corpmen under combat conditions.

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effective measures which will eventuate in vast dollar savings.

- 24. (U) To construct equipment for the synthesis and delivery of electroanalgesic currents. This will be followed by studies designed to determine optimum currents and frequencies. Methods of application and administration through time and intensity changing currents and chemical adjuncts will be studied. The final study will involve a demonstration of the safety of the use of this equipment.
- 25. (U) (76 07-77 10) Attempts have been made to minimize the discomfort elicited by the transcranial application of electroanesthesia (EA) currents and to improve the effectiveness of their delivery. Multiple miniature electrodes for use with Rhesus monkeys were designed and fabricated. Distribution of the EA waveform between two supraorbital and two occipital electrodes appeared to be ineffective in reducing induction pain. With the concomitant use of phencyclidine hydrochloride, however, the anesthetic state was produced at somewhat lower peak and average current levels than those required for previously employed electrode configurations.

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- 23. (U) To investigate the source and treatment of oro-facial infections encountered in field conditions, foreign countries and diverse climates. To evaluate the special agents, instruments and chemicals necessary under military conditions.
- 24. (U) Oro-facial infections of significance in the diverse military environment will be studied by microbiological, immunological and electronmicroscopy methods. Possible sources of oral infections will be evaluated and the effectiveness of commercially available as well as in-house designs will be studied for their ability to control or prevent oral infections.
- 25. (U) (76 07-77 10) A practical method of decontaminating the water supply of dental units and ultrasonic scalers has been devised. Continued study of the effectiveness of a SnF<sub>2</sub> mouthrinse on bacterial colonization on teeth indicates that streptococcal counts are dramatically reduced. A rapid method of bacterial identification has been successfully applied to oral streptococci. Initial experiments on in-vivo slow release of bacterial antigens from biodegradable PLA/PGA have been successful. Data has been obtained on the best method of minimizing bacterial contamination of tooth-brushes. A study of immunoglobulin levels in various gingival inflammatory processes has provided background data on the protective mechanisms in oral tissues. Data has been obtained concerning the role of oral gram negative organisms in delaying wound healing. A study of the effect of bacterial contamination beneath oral wound dressings has begun. Preliminary data of the development of more rapid means of identifying oral gram negative organisms in anaerobic wound infections has been obtained.

### IDENTIFICATION AND CONTROL OF ORO-FACIAL INFECTIONS OF MILITARY IMPORTANCE

### IMMUNOGLOBULIN LEVELS IN INFLAMED AND NORMAL GINGIVAL TISSUES

Protection of mucosal surfaces by immunoglobulins, particularly IgA is of utmost importance in preventing serious infectious diseases, many of which are acquired in tropical and foreign countries which may become combat areas. Because gingival tissue is readily accessible for study of the protective mechanisms in the tissue, findings derived from the studies of different gingival diseases may contribute to better knowledge of possible preventive and therapeutic measures in both acute and chronic infectious diseases and wound infections.

We therefore investigated the presence of immunoglobulins in inflamed and normal gingivae.

I. Immunoglobulins in Gingiva of Patients With Bone Loss of Infectious
Origin

The levels of IgA, IgG, and IgM were determined in non-inflamed and inflamed gingival tissue from patients with bone loss of infectious origin.

Maxillary and mandibular gingival biopsies were obtained from two groups of patients. The first group consisted of 12 patients (age 19-56; 8 males, 4 females) with early to moderate bone loss and pockets ranging from 4-6mm and gingival inflammation scores of 1-2 according to the Loe and Sillness Index.

The second group consisted of 11 patients (age 22-40; 9 males, 2 females) with clinically healthy gingiva requiring tissue reduction during surgical procedures.

Gingival tissue was removed after local infiltration with xylocaine hydrochloride, 2% with 1:100,000 epinephrine. Immunoglobulin concentrations

of the tissue extracts were determined using low level radial immunodiffusion plates and WHO serum standards as reference. Results for tissue from patients with moderate bone loss showed the presence of IgA and IgG in all samples over a wide range of values. In contrast IgM was detected in gingiva of only 2 out of 12 patients. The IgA and IgG values for normal and diseased gingiva did not differ significantly. IgM was found in the normal gingiva of 5 subjects. The calculated IgG/IgA ratios in normal and inflamed gingiva varied markedly among patients in both groups.

The findings of this study indicate that both IgG and IgA are present in gingival tissue and that their levels in normal and diseased gingival tissues do not differ significantly. Also it appears that IgM is detected less frequently in moderately inflamed tissue.

# II. Immunoglobulins in Granulation Tissue of Infectious and Non Infectious Type Bone Loss

The concentrations of IgA, IgG, and IgM in the granulation tissue removed from deep infrabony pockets, greater than 8 mm, was determined in 15 specimens from 5 patients (ages 12 to 20) with non-infectious bone loss and 14 specimens from 12 patients (ages 20 to 63) with bone loss of infectious origin.

Immunoglobulin concentrations of the tissue extracts were determined as mentioned above.

Results for both groups of patients showed a wide range of values. However the preliminary data showed that non-infectious granulation tissue had 1.77 times more IgA and 1.53 times more IgG than infected granulation tissue, with little difference in IgM concentrations.

These data seem to indicate that a difference exists in the granulation tissues of non-infected and infected bone loss. Of interest also is that

tissue samples removed from different defects of the same non-infected patient revealed different immunoglobulin levels.

### III. Immunoglobulin Levels in Drug Induced Hyperplastic Gingival Tissue

Alteration of serum immunoglobulin levels attributed to hydantoin drugs has been reproted to result in reduction of serum IgA in some patients and elevation in others. It was the purpose of this study to quantitatively compare immunoglobulin levels in phenytoin hyperplastic tissue (8 patients) to both normal gingiva (11 patients) and idiopathic gingival hyperplasia (7 patients). Twenty-five percent of the phenytoin hyperplastic gingiva assayed showed IgA levels above the range for normal tissues and 25% showed levels below that range. Levels of IgM were elevated in the phenytoin patients and were detected in approximately 85% of the patients sampled as compared to detection in 45% of the normal and 42% of idiopathic hyperplastic gingiva. No significant differences in IgG levels were detected between tissue types. These findings appear to be consistent with data reported on effects of hydantoins and their derivatives on serum immunoglobulins.

### EFFECT OF SnF<sub>2</sub> MOUTHRINSE ON INITIAL BACTERIAL COLONIZATION OF TOOTH ENAMEL

Our previously reported transmission and scanning electron microscopic studies evaluating the effect of flouride mouthrinses on the bacterial colonization of tooth enamel indicated that stannous flouride was an effective plaque inhibiting agent. This would be of considerable value to the combat soldier in maintaining oral health.

The purpose of this investigation was to confirm our electron microscopic findings and to further examine by microbiological methods the effect of SnF<sub>2</sub> mouthrinse on initial plaque development <u>in vivo</u>.

Cylinders of human surface enamel were embedded in Hawley appliances

and appliances were worn for 48 hours. During this time either an experimental or control one minute mouthrinse was used twice a day, just after rising and just before retiring. The experimental mouthrinse consisted of an aqueous solution of stannous flouride (100 ppmF). Tap water from flouridated water supplies (1 ppm F) was used as a control rinse. After the two day periods the cylinders were removed and processed for microbiologic and scanning electron microscopic evaluation.

Following SnF2 mouthrinsing, the total bacterial counts on enamel cylinders were reduced up to 98%, and streptococcal counts decreased up to 97.9%. It was noted that some streptococcal species appeared to be affected by SnF2 treatment to a different degree. Decrease in numbers of S. sanguis was more pronounced than decrease of other streptococcal species. Scanning electron microscopy confirmed a marked decrease of bacteria on enamel with use of SnF2 mouthrinse. It appears that in addition to reducing enamel solubility and other properties, SnF2 may also alter bacterial accumulations on teeth. Such a mouthrinse available during periods of committeent would markedly decrease dental emergencies. Further studies are planned.

## MICROBIAL CONTAMINATION OF DENTAL UNITS AND ULTRASONIC SCALERS

The microbial contamination of water systems in dental units and ultrasonic scalers has been previously investigated and demonstrated by this Institute. The infectious potential of contaminated water, to dentists, patients and particularly compromised hosts, has been shown. Partial decontamination through flushing procedures has been demonstrated. This is an especially important problem to the military where contamination via the water supply can spread among several units.

Current study has investigated practical filtration methods for decontamination of the water from ultrasonic scalers and high speed handpieces.

3.0µm pore size pleated membrane capsule filters have been used as primary filters, and 0.45µm pore size sterile disposable cellulose acetate filter units have been used as secondary, or final, filters.

Our studies have shown that after waterline sterilization, the use of a sterile, disposable, membrane filter can eliminate the microflora from the water of an ultrasonic scaler for up to 48 hours. Similarly, the water from a high speed handpiece can remain bacteria free for up to 72 hours when a 0.45 m pore size membrane filter is installed into the waterline. This system of decontamination by filtration presents a workable approach to the problem of contaminated water, and merits further research and development.

Development of uncontaminated water systems in dental equipment may result in a decreased incidence of post-operative infections and a quicker return to duty for soldiers undergoing dental treatment. Further if large quantities of water can be rapidly decontaminated by these methods it will have important affects on general water treatment procedure during periods of field committment.

#### A SEROLOGIC INVESTIGATION OF EARLY WOUND HEALING PROCESSES IN THE PRESENCE OF ORAL GRAM NEGATIVE ANAEROBES

Certain bacterial polymers may be capable of activating the complement system during the early phase of wound healing by a route other than the classical pathway. The resulting prolonged release of biologically active phlogistic factors in combat wounds contaminated with oral bacteria could

have a delaying effect upon normal healing and could be important in the pathogenesis of oral wound infections.

Some oral gram negative anaerobic forms, members of the <u>Bacteriodaceae</u>, are pathogenic in man, have been isolated from water, soil, feces, body cavities, and primate gastrointestinal tracts and are therefore highly probable contaminants of combat wounds. The purpose of this study is to investigate the potential of bacteriodaceae for delaying wound healing via alternative pathway complement activation.

Using the divalent chelators, EDTA and EGTA, it is possible to capitalize upon the specific divalent cation dependency (magnesium) of the alternative pathway in order to separate its activation from the calcium dependent classical pathway. Our results to date relate to studies in the guinea pig complement system which were undertaken to examine the feasibility of using chelators to investigate the alternative pathway activation by <a href="Bacteriodaceae">Bacteriodaceae</a> in human sera. The data shows:

- 1. Two members of the <u>Bacteriodaceae</u>; <u>Fusobacterium nucleatum</u> and <u>Leptotrichia buccalis</u>, are capable of activating the alternative complement pathway in guinea pig sera.
- 2. The factors responsible for the alternative pathway activity can be isolated in the cell walls of the organisms. The heat resistance of these factors suggests that they are polysaccharide in nature.
- Lipopolysaccharide preparations (endotoxin) from these organisms are less potent on a per weight basis than the whole cell or the cell wall preparation.
- 4. The strict alternative pathway activity of these organisms in guinea pig sera has been confirmed through the use of C4 Deficient sera.

- 5. The divalent cation chelator EDTA will inhibit both the calcium dependent classical pathway and the magnesium dependent alternative pathway in guinea pig sera.
- 6. The divalent cation chelator EGTA will inhibit the classical pathway and permit full activity of the alternative pathway in guinea pig sera. This is consistent with reports in the literature that EDTA binds both calcium and mangesium to nearly the same degree, but that EGTA shows a high binding affinity for calcium but not magnesium.

EGTA and EDTA are now being used to investigate the complement activity of six different species of oral <u>Bacteroidaceae</u> and tissue fluids. Should these organisms prove significant in the delay of combat wound healing methods for their rapid identification and elimination from such wounds will be developed.

### THE MICROBIOTA BETWEEN SURGICAL WOUNDS AND DRESSINGS

The bacterial contamination of wounds often occurs and results in delayed epithelial and connective tissue healing. Maxillofacial surgical procedures are certain to suffer some degree of wound contamination by oral microflora and may be affected by exogenous microorganisms. To determine the effects of the flora on healing wounds the organisms must first be identified. The identification and control of these microorganisms could lead to shorter healing times and quicker return to duty for the soldier who is the recipient of either elective of compulsory surgical procedures which require surgical site coverage.

The characterization of the microbiota beneath dressings has begun with the exploration of methods of disaggregation of the bacteria. The most commonly used methods of dispersion have included the use of the vortex, chelaging agents, and the use of ultrasound. Current studies under this protocol appear to show no apparent increase in the recovery rates of bacteria when either .001 M EDTA, .01 M EDTA, .1 M EDTA or no EDTA is used in the transport media. Significant differences in organism dispersion and recovery rates have not yet been found with the use of ultrasonic dispersion. The use of EDTA combined with ultrasound will also be tried prior to microorganism speciation attempts.

### COMPARISON OF MICROMETHOD SYSTEMS WITH CONVENTIONAL MEDIA FOR IDENTIFICATION OF ORAL STREPTOCCCCI

Two micromethod bacterial differentiation systems (API, Analytab Products, Inc., Minitek-Bioquest) were compared with conventionally prepared aerobic and anaerobic tubed media for the identification of facultative streptococci. A total of 14 ATCC and CSC streptococci including <u>S. sanguis</u>, <u>S. mitis</u>, <u>S. mutans</u>, <u>S. salivarius</u>, <u>S. milleri</u> and the common wound infecting organisms <u>S. pyogenes</u> and <u>S. Faccalis</u> were examined.

There was 100% correlation for all 18 carbohydrates tested. Results of this preliminary study indicate that cost and time effective miniaturized rapid bacterial differentiation systems initially developed for Enterobacteriaceae may be successfully used for physiological classification of Streptococci.

Since rapid identification of pathogenic bacteria is essential for diagnosis and treatment of many combat type diseases and wound infections, and conventional methods for identification of pathogens requires cumbersome premade glass tubes of media which requires large areas of refrigerated storage space, the miniaturized micromethod examined in this study offers a more cost effective, portable, labor saving system uniquely adaptable for field conditions.

#### SLOW RELEASE OF ANTIGEN FROM BIODEGRADABLE PLA-PGA POLYMER

Biodegradable polymer polylactic-polyglycolic acid is being investigated by USAIDR as a vehicle for the slow release of vaccines. Successful development of this modality would permit single long term immunization or desensitization treatment of the combat soldier prior to placement in a combat area or during the early period of committment. This would eliminate the additional requirement for critical services and facilities in the combat area and free the combat soldier from that dependence.

Methodology for the incorporation of Group A streptococci into the polymer has resulted in production of low titer specific agglutinating antibody to group A streptococci. Disks of polymer containing lyophilized Group A streptococci were embedded under the skin of rabbits. Antibody titers appeared approximately 30 days following implantation of a 50-50 (PIA-PGA) vaccine disk. Animals which received flakes of 100% PIA appeared to respond at 50 days whereas antibody was not detectable in animals receiving vaccine disks of 100% PIA.

Additional work is being done to determine the physical characteristics of the polymer-Ag complex necessary to get maximum immune response and to compare adjuvant activity, titer, and titer duration resulting from polymer-implanted vaccine versus routine vaccination.

# THE SEROLOGIC DIAGNOSIS OF ORAL GRAM NEGATIVE BACTERIODACEAE IN ANAEROBIC WOUND INFECTIONS

Members of the Bacteriodaceae are pathogenic in man and have been isolated from the blood following dental procedures, from lung abscesses, from upper respiratory tract infections, from brain abscesses, from surgical wound infections, and from cases of purulent sinusitis. In the past,

the rapid identification of oral gram negative anaerobic organisms in wounds of the oro-pharynx has been limited by the fastidious growth characteristics of this group of organisms. In addition, the techniques and equipment necessary for clinical isolation have physically restricted the field mobility of diagnostic laboratory facilities. Any delay or failure in bacterial identification will ultimately postpone the institution of proper therapy, prolong treatment time, and delay the return to duty of essential military personnel.

The object of this study is to develop group and type specific immunoflourescent assays from hyperimmune rabbit antisera produced against cell
wall and cytoplasmic antigens from strains of <u>Bacteriodes</u>, <u>Fusobacterium</u>,
and <u>Leptotrichia</u>. It is anticipated that accurate sero-diagnosis could be
accomplished in the field within 1-3 hours. In support of the USAIDR
mission, this study is also designed to provide militarily relevant information on the etiology, epidemiology, and prevention of wound infections by
oral gram negative anaerobic bacteria.

To date four (4) prototype organisms have been grown anaerobically in broth cultures and their identity confirmed. Preparations of cell walls and soluble antigens have been made and thus far five New Zealand white rabbits have been immunized with these preparations.

### AN INVESTIGATION OF THE TOOTHBRUSH AS A CARRIER OF BACTERIA

The infection of maxillofacial wounds and of oral surgical sites continues to be a problem in military oral surgical practice. In an attempt to decrease the incidence of post-operative infections of maxillofacial wounds and oral surgical sites, a study of toothbrush contamination was performed. Results of this study showed that fewer viable organisms were found on the toothbrushes hanging in air and that sterile toothbrushes

were better protected from bacterial contamination by test containers than by conventional containers. Air storage is recommended in normal environments and containerized storage in contaminated environments, such as field conditions or shared hospital rooms.

Further studies were held in suspense pending manufacturers delivery of toothbrushes of innovative bristle shapes, designed to permit faster drying and bacterial death. No further progress has been made due to non-delivery of test brushes. This study emphasizes the potential benefits of the toothbrush inclusion into the Army ration being developed at USAIDR.

### A MODEL FOR THE STUDY OF CANDIDA ALBICANS CAUSED ORAL INFECTIONS

Acute radiation reaction of the oropharyngeal mucosa is often painful, results in weight loss and interrupts therapy. One of the primary causes of the mucositis and pain is secondary infection by normal oral flora; especially candida. Similarly candida is a major problem in individuals with full and partial dentures. Thirty percent of the military population wear dentures.

The objectives of the study were to:

- a. Determine the presence of Candida Albicans and investigate methods of eliminating it through treatment or prevention.
- b. Determine factors such as pH which may influence Candida shift to a pathogen.

Seven patients have been followed from pre-radiation through radiation treatment. These are the findings:

a. Radiation to other than head and neck gave no pH change on teeth and oral mucosa.

- b. The oral pH of patients receiving head and neck radiation decreased during treatment but returned to normal post treatment.
- c. The oral pH of patients receiving head and neck radiation and surgery decreased and did not return to normal.
  - d. Gingival bleeding index did not change.
  - e. Radiation mucositis was not complicated by an increase in Candida.
  - f. Radiation caries was controlled by SnF2; an important finding.

It is concluded that although many reports state candida proliferates in the oral cavity and becomes pathogenic post radiation, this study did not show an effect.

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(U) Internal-External personalities (U) Oral Hygiene (U) plaque scores (II) personality types.

- (U) Under Title X of the Military Code, the Army is authorized to routinely provide extensive and effective, quality dental treatment to the soldier. However, since the possibility of conflict is ever present, combat readiness is stressed via delivery systems which bring dentistry to the soldier to assure his oral state does not preclude performance of duty. This is different from the civilian system where the patient seeks treatment. It should be noted however, that no matter what delivery system is utilized it is neither comprehensive nor effective enough to assure compliance by the patient. Factors must be identified which will motivate the soldier to initiating, accepting, and maintaining dental treatment and thereby eliminating potential periods of ineffectiveness and lost duty time. These same factors will also enhance professional productivity and reduce greatly the treatment costs.
- (U) Identify personality components unique to military patients which have a direct influence on treatment outcome. Identify which patients will respond to given methods of influencing preventive dental care. Identify those occupational factors which affect dental health care delivery by dental personnel. Identify those educational factors which lead to the best oral health care delivery.
- 25. (U) (76 07-77 10) A study was done on the effect of an educational presentation on oral hygiene scores of personality types labelled internal and external. It was hypothesized that internals should respond more positively to efforts to influence their hygiene scores than externals. The hypothesis was not confirmed and it is suggested that the mode of presentation did not provide sufficient stress to interact with personality type to accomplish the change desired.

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(U) Osteoclast Activating Factor; (U) Lymphocytes (U) Macrophages; (U) Osteoclasts

- 23. (U) To establish the precise role of human lymphoctyes and macrophages in the generation of the bone resorber, Osteoclast Activating Factor (OAF), and also to determine characteristics of the generated OAF product.
- 24. (U) Functinoal relationships between subpopulations of human mononuclear leukocytes (T and B-cells, and macrophages), with and without stimulation, will be systematically explored for their ability to produce OAF. Also, various physiochemical and immunological techniques as well as enzymes and inhibitors of known bone resorbing agents will be utilized to determine the specificity of OAF.
- 25. (U) (76 11-77 10) Generation and purification methods were developed for the isolation of the bone resorbing activity, OAF. Human MIC was used to generate large quantities of OAF. Elution of this bone resorbing activity from Sephadex G-75 chromatography was with molecules between 12,000 and 14,500 Daltons. A highly potent preparation could be obtained following concentration and re-cycling over a Sephadex G-75 superfine column. The activity was found not to adsorb to resin in anionic exchange chromatography. Anodol migration of OAF was noted in preparative AGE from which the OAF activity could be eluted.

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(U) Fixation Devices: (U) Shape Recovery

- 23. (U) To exploit the shape memory phenomenon of 55-Nitinol for enhancement of the treatment capabilities of the Army dentist and physician. Realization of the conceptual uses of this unusual metal will result in substantial savings of laboratory costs and professional man-hours.
- 24. (U) To design, fabricate, and test by means of animal and human studies, the following devices; (1) flexible wire clasps that will withstand accidental deformation out of the mouth, yet recover in the mouth; (2) prestressed surgical fixation staples and plates that will bend or contract slightly at body temperature, bringing bone fragments into close approximation or under slight compression; (3) self-anchoring fixation pins and endosseous implant devices; (4) collapsible devices for placement into defects (cyst cavity, cleft palate, etc) through orifices smaller than the inside diameter; (5) fixed and removable prosthetic appliances, restorations or precision attachments that can move into undercuts in the mouth.
- 25. (U) (76 07-77 10) Experiments have shown that the shape recovery of the test nitinol wire at body temperature is not complete. Shape recovery, depending upon the diameter of the wrought material, can vary from 80 to 90 percent. Abruptness of the recovery, however, has necessitated the design and development of miniature springs to minimize recovery forces. It is anticipated that the new innovation will preclude the dislogement of memory programmed fixation devices from osseous tissues upon shape recovery. Animal studies are planned.

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12. TECHNICAL OBJECTIVE, 2. APPROACH, 28. PROGRESS (Pumids Individual paragraphs Identified by number. Proceeds test of each with Security Closeffication

23. (U) To develop new and simplified methods of preventing oral diseases and maxillofacial injuries. To assess new methods of (1) improving the biologic management of

- facial injuries. To assess new methods of (1) improving the biologic management of militarily relevant oral conditions and (2) improving the cost-effectiveness factors of preventive dental therapy in the military.
- 24. (U) Studies will be conducted on military installations which will evaluate (1) methods of prevention of militarily relevant abnormalities and maxillofacial injuries; (2) methods of improving preventive dentistry delivery systems; (3) methods of improving cost-benefit ratios concerning delivery of preventive dentistry as a consequence of military duty; and (4) investigate the various hazards involved in the Army dental health delivery system.
- 25. (U) (76 07-77 10) A survey of accidental dento-facial injuries among Army personnel at 15 posts showed an incidence of 379 injuries per 100,000 soldiers or 2,896 injuries per year. Dental treatment time was estimated at 10,281 hours. A study of noise levels in Army dental clinics and laboratories showed that a potential hazard exists and should be further investigated. A spoon-toothbrush combination is being evaluated for field use as a means of encouraging oral hygiene. Initial results indicate excellent acceptance by the solider. A faucet operated pulsating water irrigation device is being evaluated against its motor driven counterpart. A dental instrument to facilitate mass dental care in the field is in the design stage. A rapid method of evaluating troop oral health in the field has been tested and the results are being analyzed. A study has begun on the cleaning efficacy of non wettable polyester bristles in toothbrushes.

#### PREVENTIVE DENTISTRY MEASURES OF MILITARY SIGNIFICANCE

### AN EPIDEMIOLOGICAL SURVEY OF ACCIDENTAL DENTO-FACIAL INJURIES INCURRED AMONG ACTIVE DUTY ARMY PERSONNEL

Fifteen representative Army posts were selected as study sites so as to provide a complete range of military functions. They are categorized as being primarily either combat arms, combat support, combat service support, basic training, aviation or headquarters posts. The combined average census of the 15 posts during the study period was 129,191, or approximately 25 percent of the active Army strength. For the time period September 1975 - August of 1976 a total of 747 accidents were reported. Analysis of the data during that period shows the following: fifty-one percent of the injuries occurred at combat arms posts and 31 percent at basic training installations.

Using age group as a criterion, 56.5 percent of the accidents occurred in the 17 - 20 year old group; by military rank, 32.7 percent were E-1; considering years in service, 59.8 percent had one year or less; and with respect to highest education level, high school graduates were involved in 51.8 percent of the trauma.

Fist fights and sports-related injuries accounted for 29.7 and 20.3 percent of the trauma, respectively, by type of sport, 48.3 percent of the injuries occurred while participating in football and 21.9 percent while playing basketball. More times than not the sport was military sponsored, with many injuries incurred during "combat" versions of football and basketball. Mouthguards were available in only 5.9 percent of the instances and in use in a mere 3.3 percent.

A distinction was made between time to treat the emergency condition itself and time required for follow-up treatment. 85.1 percent of the cases took up to an hour to treat initially. Succeeding care required up to 1110 minutes to complete, with a mean of 172 minutes.

The dependent variable - dentofacial injuries - was categorized by
the nature of the injury sustained, e.g., soft versus hard tissue involvement
region of the oral cavity involved, fracture type, and so forth. The independent variables were 56 in number and included general and demographic
information, as well as detailed data on the history of the accident.
Statistically significant relationships between independent variables such as
first fights, sports, vehicles, weapons, and other pieces of equipment
and the dependent variable were found.

The dentofacial accident incidence rate in this study group of 15 representative Army posts was 379 per 100,000. This rate extrapolates to an Army-wide number of 2,896 cases per year. Using a mean combined treatment time of 213 minutes per case, approximately 10,281 hours might be expended annually by the Army Dental Corps on dentofacial accidents. This is based upon the assumption that the reporting has been complete and accurate and that the study group was truly representative of the entire Army.

It was noted that 20 percent of the trauma resulted from sports and that 70 percent of these injuries occurred while participating in football and basketball. If this damage could be prevented through the use of mouth-guards while playing these two sports, the Army could possible convert some 180 dentist-days of emergency treatment time to definitive care. It seems likely, therefore, that a satisfactory cost-benefit ratio might be achieved through the fabrication and mandatory use of protective mouthguards by personnel participating in Army-sponsored contact sports.

To determine if the variation noted from month to month might be seasonal, this study was extended for a second year. Due to personnel turbulence within the Division and at many of the study sites, a delay was experienced in reinstating this study. One hundred and seventy-four accidents have been reported to date, however, further time must be allowed to permit comparison of this data with the preceding time frame.

### AN EPIDEMIOLOGICAL STUDY OF NOISE LEVELS U.S. ARMY DENTAL CLINICS AND LABORATORIES

Based on the findings of a recent survey conducted by USAIDR at the annual Americal Dental Association meeting and a survey entitled "The Extent of Hearing Loss in the Army" which indicated that hearing loss could be a major health hazard in the U.S. Army, this study was initiated to determine if noise levels within dental operatories and laboratories posed a potential threat to the auditory apparatus.

An initial survey was conducted at Fort Rucker, Alabama because of the potential additive affect of aircraft noise. Three clinics were surveyed. Noise levels were taken 15 inches form the noise source. These sources included laboratory grinding machines, various handpieces, and suction apparatuses. Two cast grinding machines were found to produce levels of 83 and 80 dB's when in the process of trimming casts.

Measurements were taken on four separate high volume suction apparatases. These gave readings of 84, 70, 74, and 71 dB. Eight Starflite Futura handpieces were tested. These ranged from 70 dB to a high of 91 dB. The average reading was 79 dB. One such handpiece was tested when cutting in the mouth and showed a level of 80 dB. This did not alter when removed from the mouth. A single Borden Air Rotor handpiece was examined. This handpiece

produced a level of 86 dB with a peak of 93 dB. One Starflite handpiece gave a reading of 66 dB. This was an older model than the Futura and had a lower r.p.m. rating.

The Starflite Futura handpiece that we found to have a noise level of 91dB was used infrequently since the dental officer using this equipment complained of the noise it was producing. The turbine was replaced and when retested showed only a 66 dB reading.

This study is being continued to include a wider range of clinics.

The finding that many handpieces produce a noise level which approaches the limit considered acceptable for an eight hour exposure, i.e. 85 dB, and in some instances found to reach levels only acceptable for four hours, i.e. 90 dB, indicates the need for such continuation.

Inasmuch as the military dentist is exposed not only to the noise levels described, but to additional exposure from aircraft, artillery, and small arm fire, and inasmuch as such exposures may be accumulative there is a potential threat to hearing loss and consequent decreased military duty performance.

### DEVELOPMENT, TEST AND EVALUATION OF A SPOON-TOOTHBRUSH COMBINATION FOR FIELD USE

Inasmuch as the oral health of the combat soldier is a constant problem and a frequently received complaint is that there is not time or toothbrush available when in the field, this study is an attempt to provide a toothbrush which is available when eating and to determine if this will increase the overall oral health of those to whom such is made available.

An initial study was conducted using a unit assigned for approximately one month to a field exercise. A prototype spoon-toothbrush was provided

two weeks a survey was conducted to determine, by means of a questionnaire, if the spoon-toothbrush combination had been used, if the field soldier felt it was valuable, and if any problems had developed regarding the prototype. Eighty-two questionnaires were received. Results indicated that 92% picked up the spoon-brush, and of these 87% used the brush. Ninety-three percent of the respondents indicated they would use the spoon-brush combination if it was made available to them on a continuing basis when in the field. Acceptance of this mode of supplying tooth brushes in every field ration would decrease the high time loss in field committed soldiers attributed to dental disease.

### EVALUATION OF A FAUCET OPERATED PULSATING WATER IRRIGATION DEVICE (FOPWID) IN A MOTOR DRIVEN IRRIGATING DEVICE

This study was conducted to evaluate the ability of a faucet operated pulsating water irrigating device (FOPWID) to maintain or improve oral health when compared to a motor driven device (MDD), since the former is significantly cheaper. The study was conducted using 78 service academy personnel. Two indices were chosen to determine the state of oral health, the Quigley-Heine plaque index and the gingival bleeding index (GBI). There were two examiners; one performed the GBI and the other the plaque index. Following the initial examination, individuals randomly stratified in to control, FOPWID and MDD groups, each balanced with respect to GBI and plaque scores. There was an unexpected increase in the GBI of all groups and an increase in the plaque scores of the control and FOPWID groups. The negative results are attributed to factors not considered at the time of the initiation of the study. Most important was the coincidence of the experimental period with a significant

stress situation among the participants. It was concluded that the study should be repeated with a more representative military population. However, the finding of a stress related disease in oral hygiene has a high military importance.

### EVALUATION OF THE CLEANSING EFFICACY OF A POLYESTER-BRISTLED TOOTHBRUSH

Maintenance of the oral health of the combat soldier when in the field is a continuing problem. This study is designed to determine if a polyester bristled toothbrush will provide better cleansing efficacy than the standard brush of nylon. Polyester bristles are non-wetting, and should therefore retain their stiffness and further reduce the potential for microbial growth, an important factor under field conditions when toothbrushes are constantly exposed to all indigenous organisms. This protocol has just been approved. Currently the manufacturer is preparing the desired number of brushes as to bristle size and shape. Study sites are being evaluated.

A DENTAL INSTRUMENT COMBINING A CHIP BLOWER WITH VARIOUS EASILY INTERCHANGEABLE TIPS: A DESIGN TO FACILITATE MASS DENTAL CARE AND TO SIMPLIFY THE ARMAMENTARIUM FOR FIELD DENTISTRY

Military dentists can be called upon to provide mass dental examinations and to do dental treatment under circumstances demanding the utmost in efficiency with the help of only minimal resources. Instrumentation that caters to this need promises important benefits, especially in field practice. Demand on the part of busy dental officers for some method of conveniently accomplishing routine drying and debris removal during procedures in the mouth appears to center around a combination dental explorer/chip blower which could also accompdate other instrument tips as appropriate and practical. Work has continued during the past year on the design of such an instrument.

The original design in which air flow is controlled bu an elongated ball valve rocked out of its seat by pressure on its extremity, has been supplanted by a simpler, more reliable and trouble-free arrangement. The newer, pinch tube concept will be more positive in function, cheaper and less complicated to build, and will simplify any needed parts by the user. Pressure on a button in the handle will produce a fully controllable air flow and provide a positive cut-off not dependent upon precise fit of parts as would be the case with the ball valve. Instead of a screw-in, operating tip mount, a male-female ferrule with positive ball detent indexing and locking seems most practical and functional, allowing swift and convenient interchangeability at chair-side. Work continues.

#### A RAPID SCREENING TEST FOR EVALUATING TROOP ORAL HEALTH

Gingival inflammation and alveloar bone loss are the main cause of tooth loss in patients over age 35. It has been shown that gingival inflammation frequently starts at an early age and requires extensive time and expense to treat in military personnel.

A simple, objective, and inexpensive clinical test for gingival inflammation would permit rapid screening examination of soldiers for the presence of initial gingival inflammation and more severe bone loss. Those soldiers giving positive results could then be identified and thoroughly examined to determine the extent of pathology present.

Such a test should provide reliable and valid diagnostic results with a minimum expenditure of time, materials, and personnel resources. Fluctuations with regard to subjective examiner error would be kept to a minimum. The object of this study was to devise such a test.

Patients receiving oral hygiene instruction (OHI) at an established dental clinic were utilized for this study. Subjects were tested before, during and after the OHI series. Each subject was examined for gingival inflammation using the Gingival Index (G.I.) of Loe and Silness, to determine which group the subject belonged to so that correlations with other parameters. could be done. At the start of the study a two minute salivary sample was collected from the subject in screw-top scintillation vials and the volume was recorded. The sample was immediately tested using a Hema-Combi-Stix (HCS) to assay for the presence of blood and protein. Each subject was then asked to brush his teeth for one minute using the standard issue tooth brush which had been wet with plain water. After brushing, the subject rinsed with 20ml of distilled water, which was collected for analysis in a labled scintillation vial. The tooth brush itself was rinsed in 10 ml distilled water in a separate scintillation vial. The water in both vials was tested for hemoglobin and protein using the (HCS). The samples were then frozen for laboratory analysis. Quantitative hemoglobin and protein levels were determined on all samples for comparison with the HCS and G.I. results in order to determine if hemoglobin and protein levels using HCS is a valid test for gingival inflammation.

All samples have been collected and biochemical analysis has been done. Statistical analysis of the results is now under way.

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23. (U) To determine the feasibility of the application of laser technology to prosthetic rehabilitation and to maxillofacial wound debridement and treatment. Utilization of this technology if proven successful could save precious metal costs of up to \$1,000,000 per year.

(U) Neodymium Laser; (U) Ruby Laser

- 24. (U) Energy levels, methods of contour and approximation of pontics to establish optimum weld patterns and strengths will be investigated. This will be accomplished first in a bench set-up and secondly in animals to establish feasibility and safety. To expand the capability via the continuous wave carbon dioxide laser for use in maxillofacial surgery. A comparison of healing of laser vs. present surgical techniques will be evaluated for speed, esthetic results, secondary infectious complications, blood loss and ease of use.
- (U) (76 07-77 10) Studies on the feasibility of in-vivo laser welding of fixed prosthodontic appliances are continuing. Dental pulp appears to be sufficiently resistant to the Neodymium laser to permit this application. It was found that tooth surfaces could be "glazed" by laser radiation while implanted control surfaces were unaffected by the same energy level. "Glazing" is reported to provide protection against caries. A study of the effect of neodymium and ruby laser radiation on dental pulp showed that the ruby laser was more damaging to pulp. Energy levels up to 1959 Joules/cm<sup>2</sup> did not effect pulp when neodymium radiation was used.

#### APPLICATION OF LASER TECHNOLOGY TO MILITARY DENTISTRY

#### IN-VIVO LASER WELDING

Two reports published by this laboratory in 1977 have demonstrated that the pulp is resistant to injury by the neodymium laser and in vitro laser welding of dental alloys is possible. We are presently investigating the possibility of in-vivo welding of fixed prosthodontic units. One animal was completed last year and the results suggested that the dental pulp was sufficiently resistant to Nd laser radiation so that five additional animals are now currently under investigation. Both short and long term pulpal response to in-vivo welding will be evaluated during this portion of the investigation. Similar crown preparations and welding techniques are being used but three of the animals will be followed for up to three months to evaluate the long term pulpal response. If the additional investigations support our previous findings, human use studies of welding of prosthodontic appliances and fracture fixation devices may be attempted with an anticipated savings of both time and material.

#### LASER EFFECTS ON ORAL TISSUES

Previous reports from our laboratory and others have indicated the possibility of using the laser for the "glazing" of enamel as a means of prevention of dental caries. Our laboratory is also in the process of evaluation of electroless metal plating of teeth as a preventive measure. A preliminary study combining these two systems was initiated by our laboratory.

Two human molar teeth, one silver plated and one control (unplated)
were subjected to similar amounts of neodymium (Nd) laser radiation and then
studied with the SEM to evaluate the effects.

The results of the SEM studies demonstrated that with essentially equal

energy densities, the control tooth surfaces (3) showed no effects of the laser but the silver plated surfaces (3) demonstrated a "glazing" effect. This "glazing" effect is reported in the literature to provide a decreased susceptability to both in vivo and in vitro subsurface demineralization (early caries).

#### DENTAL PULP EFFECTS OF NEODYMIUM LASER

The neodymium (Nd) laser has been demonstrated in our laboratory to be more efficient than the ruby laser in the in vitro welding of dental materials. The purpose of this study was to determine the effects of the Nd laser on the dental pulp.

A total of eight teeth from 2 Rhesus monkeys were exposed to various amounts of Neodymium laser radiation. Energy levels up to 1959 Joules/cm<sup>2</sup> did not produce any deviation in the normal histology of the pulp. Evaluation of the results of this study produced the following conclusions.

- The response of the dental pulp to the Nd laser is much less severe than its response to ruby laser radiation when equal energy densities are considered.
- 2. The pulpal response to the Nd laser is of a different character than the pulp response to the ruby laser. The Nd laser response is focal and confined to the effected dentinal tubules. The ruby response is focal and in addition, has a hemorrhagic effect on the central pulpal vessals which appears to trigger a more generalized degenerative change in the pulp.
- 3. These preliminary results clearly suggest that the pulp is more resistant to injury by the Nd laser than by the ruby laser. Further testing of these findings are continuing. If subsequent results support these early findings, reevaluation of the use of the neodymium laser in vital tooth procedures would be definitely indicated.

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23. (U) To evaluate new materials and material of special interest to the Army dentist Criteria for selection of materials, devices or techniques for evaluation are based on anticipated high potentials for: (1) savings of fiscal and/or manpower resources; (2) work simplification; (3) improved health care delivery in combat areas; and

(4) enhanced safety with respect to professional and ancillary personnel as well as to the patient.

- (U) New materials will be evaluated on the basis of the following parameters; Composition, microstructure, physical and mechanical properties, cytotoxicity, and clinical performance.
- (U) (76 07-77 10) Developmental improvement and clinical evaluation of the USAIDR pulp tester continues. Continuing evaluations of commercial "high-copper" amalgam alloys are serving as a basis for developmental and clinical studies. The development of an agent for enhancing porcelain-alloy bond strength was completed. Evaluations of resin and gypsum based die materials indicate that the resins are unsuitable for Army use. Asbestos hazards in an Army dental lab have been studied. A clinical fit comparison of removable partial dentures made by different methods has been completed. A number of new alloys have been evaluated for Army use. Studies of new impression materials and the tissue response of base metal crown and bridge alloys have begun. Cavit has been found muitable as a temporary filler for vital teeth. SEM and microprobe studies of dental cutting instruments and electroless plated teeth have been done.

# DEVELOPMENT AND EVALUATION OF DENTAL MATERIALS AND MATERIEL FOR ARMY USE

#### IMPROVEMENT OF VISCOELASTIC PROPERTIES OF DENTAL RESTORATIVE MATERIALS

Stress relaxation studies on dental amalgam have been continued.

Materials considered within the present reporting period include five
newly marketed "high-copper" alloys, indiloy, Aristaloy-CR, Cupralloy,
Tytin and Sybraloy. Stress relaxation which follows deformation induced by
transient masticatory forces or thermal expansion and contraction provides
a means by which clinically observable opening of the tooth-amalgam margin
may occur. Thus, the use of tooth restoratives which exhibit minimal stress
loss in function is understandably desirable.

Stress relaxation of the subject materials was low and comparable to that of a widely used dispersed-high copper phase system, Dispersalloy. It is likely that alteration of alloy particle morphology and phase distribution would further reduce stress relaxation of high-copper amalgamates. The development of technology to achieve this goal is now in progress. This is militarily highly important because it has been shown that 25% of amalgam fillings fail within 5 years and 3% fail within 2 years.

#### PORCELAIN-ALLOY BOND STRENGTHS

A coating agent for enhancement of alloy-porcelain bond strength has been developed. This development provides a composition of matter comprising substantially one part by weight fine powder aluminum, substantially 5 parts by weight opaque dental porcelain, and sufficient water to form a useable aqueous slurry. The composition may include ethyl alcohol or any other liquid which will promote wetting of the substrate surface by the aluminum ceramic admixture.

Improved base metal to porcelain bonding that can be attained through the use of the aluminum-ceramic coating material broadens the range of application of inexpensive nickel-chromium alloys in military dental practice. The advantages of this coating composition over prior art products are its low cost, ease of preparation and manipulation, and effectiveness in promotion of alloy-porcelain bonding. A patent disclosure has been filed.

#### LABORATORY EVALUATION OF DENTAL AMALGAM ALLOYS

Five newly developed high-copper amalgam alloys were evaluated in a laboratory study to determine their suitability for use in military dentistry. Measured property values of these materials were compared to those of eight conventional alloys which presently enjoy wide usage in the dental clinics of the United States Uniformed Federal Services.

Setting expansion or contraction was a characteristic feature of the amalgamates. Ten of 13 alloys exhibited contraction, whereas 3 showed expansion. Early (15 minute) tensile strengths of ternary high-copper materials were higher than those of either a dispersed phase product or the conventional alloys. Early tensile strengths ranged from 340 psi for one conventional material to 1,200 psi for an indium-containing high-copper alloy. The ranges of mean tensile and compressive strengths at 24-hours were relatively narrow. Static creep (deformation under constant load) of the high-copper alloys was low (0.21 to 0.71 percent). The laboratory data will be used to establish an appropriate human use protocol for long term clinical evaluation of the test materials.

#### EVALUATION OF RESIN DIE MATERIAL

The task was initiated to assess the potential usefulness of three newly marketed resin die materials (Epoxy Die, Epoxydent and Pri-Die) in military dental practice.

Data evaluated to date indicate that dies fabricated from the polymeric materials are more durable than those poured from densite stone. Notable, however, is the difficulty experienced in manipulating the resinous materials. Measurements for determination of dimensional accuracy indicate that resin crown—and—bridge dies are undersize. The test materials are not suitable for routine use in military dentistry. Improvement of existing formulations does not appear feasible.

CLINICAL COMPARISON OF REMOVABLE PARTIAL DENTURE FRAMEWORKS FABRICATED BY "HIGH-HEAT" AND "LOW-HEAT" LABORATORY TECHNIQUES

The following goals have been attained: (1) Parameters of apparent fit for cast removable partial denture frameworks have been established and defined; (2) comparison of the clinical fit of castings made by two techniques has been made; and (3) time required to achieve an acceptable "clinical fit" with Ticonium 100 and Vitallium frameworks has been determined.

Significant differences in the quality of castings made by the two techniques were not found. The Vitallium procedure, however, requires the use of highly technique sensitive investment materials. Fabrication of acceptable prosthetic devices from Vitallium can be achieved only through meticulous conduction of all phases of the prescribed technic. The use of beryllium containing Ticonium 100, though less cumbersome, requires establishment and enforcement of rigid standards for personal and laboratory hygiene. Within presecribed limits, both Ticonium 100 and Vitallium are suitable for

military use. Neither modification of the materials nor improvement of existing laboratory technology is required.

# IDENTIFICATION AND CHARACTERIZATION OF ASBESTOS IN THE MILITARY DENTAL ENVIRONMENT

Many daily professional activities of the dentist are based upon the preparation, direct use and mechanical alteration (grinding) of dust producing minerals. Aerosolizable substances include, but are not limited to asbestos cements, plaster and dental stone, refractory investments and the filler constituents of irreversible hydrocolloids. The potential for inhalation of airborne toxic materials by the military dentists who practice in a group situation would appear to be greater than that of his civilian counterpart.

Particulate matter (dust) found in dental laboratory areas on tables, casting machines, walls and the hands of workers were characterized by optical microscopy, SEM and x-ray microprobe analysis. Compositional and physical features of the materials studied to date parallel closely the characteristics of asbestos which is known to produce pneumoconiosis and malignancy in man. Development of safer techniques for the handling of hazardous aerosolizable substances in the military dental enfironment is now in progress.

### LABORATORY EVALUATION OF GYPSUM BASED DIE MATERIALS

The characteristics of three gypsum based die materials (Vel-Mix, Super Die and Silky-Rock) were assessed. Clinically significant differences in properties and behavior patterns were not detected. All materials possessed adequate setting times, hardness, strength and dimensional accuracy as well as comparable ability to reproduce fine surface detail. All three

materials are equally suitable for use in military dentistry. Inherent limitations imposed by the mineral gypsum have precluded significant improvement of existing die stone formulations.

### CHARACTERIZATION AND IMPROVEMENT OF NEW BASE METAL ALLOYS

Characterization studies on two potential substitues for gold casting alloys have been completed. The test materials, Neydium and Ceramalloy exhibited mechanical properties comparable to those of the more costly high fusing precious alloys. Poor casting characteristics of the alloys, however, limited their range of application in clinical practice.

Experiments have shown that clean melts of Neydium and Ceramalloy can be obtained by fusion of the alloys under a protective blanket of argon. The recently developed laboratory procedure reduces porosity and thereby aleviates numerous problems inherent to the finishing of hard and rigid cast structures.

### EVALUATION AND IMPROVEMENT OF ELASTOMETRIC IMPRESSION MATERIALS

The task was initiated in response to inquiries from field commanders and clinicians concerning the suitability of newly developed impression materials for use in military dental practice. Thirteen impression materials (five polysulfides, seven silicones and one polyether) have been selected for inclusion in the study.

Initial data indicate a wide range of performance capability among the types of materials and individual products. Analysis of the test materials for detection of lead, peroxides and other potentially toxic substances will be completed not later than 31 May 1978. It is anticipated

that minor formula modifications will make possible the military use of the test elastomers under adverse conditions of storage and field service.

ASSESSMENT OF TISSUE RESPONSE TO BASE-METAL CROWN AND BRIDGE ALLOYS

Implants of the corrosion products of two nickel-chromium alloys and an iron-chromium alloy have been placed in the subcutaneous tissues of rats. Harvesting and examination of tissues affiliated with the implanted substances have commenced. Estimated completion date of this task is 30 July 1978. The data will provide a rational basis for human use studies on long term toxicity.

EVALUATION OF PRECIOUS, SEMIPRECIOUS AND BASE METAL ALLOYS

Five products have been evaluated in the laboratory, PD and PG (American Gold Co.), Neydium-Gold, Ceramic alloy (J.M. Ney, Co.) and JPW-Porcelain White (Jensen Industries, Inc.). The composition, microstructure, mechanical properties and handling properties of the subject alloys were assessed.

PD & PG though based upon a precious metallurgical system, contain minor additions of gallium (PD) and nickel and gallium (PG). Properties of PG and PD are comparable to those of indium and tin containing high-fusing alloys used routinely in military dentistry. However, PG-porcelain and PD-porcelain bond strengths are 50 to 60 percent lower than those exhibited by other available alloy-porcelain systems. Neither PD nor PG would serve as a reliable substitute for veneerable alloys presently available through Federal Supply channels.

Composition of JPW is strikingly similar to that of Cameo, a competitive product. However, the structure of JPW is characterized by large equiaxed grains, the diameters of which exceed 50 µm. Experience has shown that grain diameters greater than 30 µm render dental alloys difficult to cast and to finish. Mechanical property data for JPW exhibit inordinately wide ranges of scatter. The findings suggest that either the alloy is highly technique sensitive or that the alloy has been manufactured under conditions of poor quality control. The properties and characteristics of devices fabricated from JPW can not be improved by modification of existing investment, burnout and centrifugal casting procedures. The replacement of high quality materials by JPW is not recommended.

Data and observations on Neydium-Gold Ceramic Alloy confirm its potential usefulness in military dental practice.

Technology required to fabricate precision castings from the alloy is presently available. If priced competitively, the substitution of Neydium-Gold Ceramic Alloy for veneerable metals used presently in the Federal Dental Laboratory system would appear appropriate.

DENTAL PULP REACTION TO CAVIT TEMPORARY FILLING MATERIAL

Cavit has been used successfully in U.S. Army Dental Clinics as a temporary stopping for endodontically treated teeth for many years. Recently, however, the manufacturer has suggested that the product may be used as a temporary filling material in vital teeth. Some subsequent reports from the dentists in the field have indicated that teeth treated in this manner become sensitive and/or painful. This investigation was undertaken to determine if the use of cavit as a temporary filling material in vital teeth by Army dentists procudes an undesirable pulpal reaction which would contraindicate this particular use of Cavit.

Seventy-two teeth were utilized on three Macaque fasicularis monkeys to evaluate pulpal response to Cavit. Odontoblastic nuclei displacement was histologically evaluated at three time periods to identify initial, early and final response.

The results of this study verify that when used in accordance with the manufacturer's directions, Cavit may be employed as a temporary restorative material for vital teeth. Use of this material will result in a saving of time for both the assistant and dentist since it requires no mixing and sets rapidly. In addition, in a combat or field situation when conditions are frequently not ideal, this material may be placed in a wet cavity preparation as a temporary restorative material in order to rapidly return the soldier to duty. This may be very advantageous because under combat conditions - dry cavity preparations may be difficult to obtain and/or maintain.

#### DEVELOPMENT OF A PULP VITALITY TESTER

The design and fabrication of less cumbersome instrumentation has served to reduce mechanical vibration and electrical background interference. Additionally, enhancement of the quality of the signal caused by pulsatile changes in blood pressure and flow velocity within the vital pulp has been made possible through afferent and efferent transmission of infrared light through small diameter fiber optic components. The improved instrumentation is ready for definitive clinical evaluation. Development of an appropriate human use protocol is in progress.

#### SCANNING ELECTRON MICROSCOPY STUDY OF KIRKLAND GINGIVECTOMY KNIVES

Scanning electron microscopy was used to examine factory-sharpened, steam autoclaved and surgically dulled Kirkland gingivectomy knives. The following observations were made:

- The optimal investigatory magnification and view of the cutting edge was at 500x from a frontal aspect.
- 2. The factory sharpened cutting edge exhibited a metallic extension (wire edge) caused by grinding in a direction away from the instrument during the sharpening process. The direction of the sharpening process ultimately determined the cutting edge morphology.
- 3. Steam autoclaving did not alter the metallic character of the factory cutting edge.
- 4. Dullness of the cutting edge was observed after surgical use on the facial or lingual gingiva in one quadrant. It was observed that the Kirkland gingivectomy knife became dull after one quadrant gingivectomy and should be resharpened at that time.

This study provided more objective data in the clinical use of the Kirkland gingivectomy knife, a knife routinely used in oral periodontal surgery. Evaluation of this cutting instrument, as supplied by the manufacturer, its changes with use, and resharpening characteristics is important to its use in repair of oral maxillofacial combat wound treatment of the military dental patient. In addition, this study serves as a reference standard for general surgical instruments used in military hospitals and decreases instrument costs by effective utilization and understanding of sharpening.

# CHARACTERIZATION OF TOOTH SURFACES AFTER ELECTROLESS PLATING - AN SEM AND X-RAY MICROPROBE ANALYSIS

Several teeth subjected to an electroless plating procedure with silver, were studied by SEM and probe analysis of the pertinent surfaces. The plating procedure represents a possible means of surface attachment of dental restorative materials in military dental patients. Quantitative EDAX analysis revealed optimal surface effects with 100/300/100 gpl's of Ag NO<sub>3</sub>, Fe SO<sub>4</sub> and NaF respectively at a pH of 6. At this pH, surface elements are in concentrations of 16, 77, 2, and 5% for calcium, silver, chlorine, and phosphorus respectively. SEM of fractured surfaces with backscattered imagery and elemental mapping show that silver particles are found between enamel prisms at a distance of 1.4 - 3.6 µm, and in cementum, 4.4 µm. Further evaluation of protective effect of this penetration should be performed.

A surface active agent, strongly adherent to the tooth could be an effective agent for large scale application of sealants and other restorative materials in the military population, allowing much more efficient dental care delivery than presently being administered.

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- 23. (U) Annual Army expenditures for previous metals utilized in the fabrication of fixed dental prostheses are in the vicinity of \$1,000,000. The cost of an equal volume of base metal alloy is \$30,000. Properties of base metal alloys indicates however, that these alloys cannot be utilized for small castings without drastic metallurgical modifications. This work is therefore being conducted to: (a) Develop heat treatment methods for controlling properties of nickel-chromium based casting alloys; (b) evaluate nickel-chromium based alloys for use in operative dentistry.
- 24. (U) The properties of nickel-chromium based alloys will be studied in details by various physical methods available in order to devise procedures which will optimize their usefulness. Any improvement obtained will be evaluated clinically.
- 25. (U) (76 07-77 10) Experiments conducted during the present reporting period have shown that accurate castings can be fabricated from some crown-and-bridge golds without the use of asbestos-ring liners. Additionally, the use of a steel-mold forming ring is not required. Adoption of the new technique by military dental laboratories would reduce significantly the potential for inhalation of toxic fibrous materials. Attempts to improve the apparent fit of base metal castings through the use of modified investment materials, by alteration of the burnout procedure and by change of sprue design have not been successful.

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- 23. (U) To recognize, characterize and develop effective therapeutic measures for those lesions and conditions which effect the soldier due to military duty. The recognition of environmental and other factors which participate in the etiology of lesions and conditions unique to the military or are causally related to military duty will enable the development of interceptive or therapeutic measures.
- 24. (U) To detect through clinical and/or microscopic observation oral lesions or a condition unique to the military population. To identify oral lesions or conditions which, though not unique to the soldier, are etiologically related to the performance of duty. Once identified the natural history including etiology, therapy, and prognosis will be established utilizing appropriate methods such as surveys, animal, and human investigations.
- 25. (U) (76 07-77 10) Significant lip pathology has been found in a continuing study among active duty personnel. A study of induced collateral circulation in traumatic injuries of the tongue suggests conservative debridement. A study of soft tissue response to commercial adhesives has been completed. Data is being analyzed. A study of DMSO as a carrier of local anesthesia to tooth pulp is in progress. A method of studying the tooth-bone interface using cryomicrotomy and the SEM has been developed. Several lesions of dental interest have been studied.

reliable to contractore upon originator's approval.

#### NATURAL HISTORY OF ORAL LESIONS

#### EPIDEMIOLOGICAL SURVEY OF LIP PATHOLOGY IN ACTIVE DUTY MILITARY PERSONNEL

During FY 77 a USAIDR research team evaluated the acute and long term effects of environmental exposure to the lips of approximately 1600 soldiers. The soldiers were examined during joint training exercises "Solid Shield" at Ft. Stewart and "Brave Shield XVI" at 29 Palms MCB. The aim of this study is to document the incidence of lip lesions as they relate to the type of duty.

Data on age, type of duty (indoor or outdoor), complexion, sex and degree of lip damage was collected on each soldier examined. A preliminary analysis of the data recorded in "Solid Shield" revealed a 5 percent incidence of acute lip changes and a 9 percent incidence of chronic lip change. In the desert environment of "Brave Shield XVI" an 18 percent incidence of acute lip lesions was noted. The most common type of acute lip lesions consisted of solar cheilosis. A complete analysis of the data is in progress.

With this high incidence a test utilizing the solar simulator will be carried out in 1978 to test the efficacy of Actinic blocking agents used by the Army. Special emphasis will be upon the effects of lip wetting, mucous and saliva as possible factors which alter the typical blocking agent.

#### THE MICROVASCULATURE OF INDUCED COLLATERAL CIRCULATION OF THE TONGUE

Maxillofacial injury occurring in military combat may compromise the blood supply on one side of the face. In many instances large tissue segments are sacrificed at triage due to the severance of local vasculature. This study was designed to determine the efficacy of the blood supply to

the tongue when the contralateral common carotid artery was ligated mimicking unilateral trauma.

A total of thirty-three (33) New Zealand White male rabbits weighing between seven and nine pounds each were utilized in this study. The right common carotid artery was exposed and ligated, and the animals were sacrificed in groups of five at a time intervals of one hour, six hours, one day, one week, and two weeks. Microfil latex was used to perfuse each animal to demonstrate the vascular pattern of the tongue. Eight animals served as controls and were perfused with Microfil without having had any previous procedure performed.

After vulcanization of the Microfil, the tongue was removed from each animal and subjected to an alcohol-methyl salicylate clearing procedure for evaluation of the vascular pattern.

Preliminary findings indicate that extremely rapid revascularization occurs following unilateral ligation and that the pattern is surprisingly variable. This indicates that debridement of certain oral structures should be very conservative when compared to other tissues.

#### SOFT TISSUE RESPONSE TO SELECTED COMMERCIAL ADHESIVES

Frequently patients attempt to self repair their prosthetic appliances with adhesives not specifically designed for intraoral use. This appears to happen with some frequency in military situations especially those involving prolonged periods of field activity. The purpose of this study is to investigate the histological response of soft tissues in contact with three commercially available adhesives: a methyl-2-cyanoacrylate compound, an epoxy resin amine compound and butyl amine cement.

Adhesive filled polyethylene filled tubes were implanted subcutaneously in the abdomen of forty-eight rats. Animals were sacrificed at intervals of from four to twenty-four days, implants recovered and submitted for histological preparation.

Histological analysis of the tissue relative to inflammatory response occurring around the ends and shaft of the implants is now being completed. Preliminary results indicated relatively little histological response to the epoxy resin, and butyl amine cement but a rather severe response to the cyanoacrylate.

# RAPID, INSTRUMENT FREE LOCAL ANESTHESIA OF THE DENTAL PULP TO FACILITATE TREATMENT OF COMBAT INJURIES OR EMERGENCIES

Dimethyl sulfoxide (DMSO) is an organic solvent with the remarkable ability to cross the dermal barrier rapidly in high concentration and with little or no permanent tissue damage. It has been demonstrated to be bacteriocidal and fungicidal in vivo and in vitro. In combination with Tetracaine it produces intense and unquestionable anesthesia of the skin. It has been used with numerous other medications to provide a vehicle for application of these drugs through the skin. In view of the penetrating and transporting ability of DMSO this investigation is in progress to determine if an anesthetic agent (lidocaine) can be applied to the dental pulp through intact tooth structure.

An initial study to determine the ability of DMSO alone to penetrate intact teeth was done on 10 freshly extracted human teeth. The teeth were thoroughly washed in saline and dried. The roots were removed and each tooth was placed, occlusal surface down, in a solution containing 70% labeled DMSO

(methyl<sup>3</sup>H) and 30% distilled water. The teeth were not emersed beyond the dentinoenamel junction. Physiological saline was added to the exposed pulp chambers at the time the teeth were emersed in the DMSO/H<sub>2</sub>O solution and aliquots were removed at selected time intervals for tritium counting by scintillation spectrometry. The data indicated that DMSO penetrated the intact tooth into the pulp chamber within 15 minutes. Labeled DMSO-Lidocaine combinations will be evaluated next in the same in-vitro system described above. This method of controlling dental pain could be used by non-dental personnel in the field for pain control.

#### FIBROUS DYSPLASIA OF THE MANDIBLE AND SPHENOID BONES

A case of fibrous dysplasia of the mandible was studied in which further diagnostic procedures revealed the presence of a second and potentially more dangerous lesion. The literature was reviewed with the purpose of bringing attention to the still confused state of fibro-osseous lesions of the jaw and skeleton. The relationship of the ossifying fibroma and fibrous dysplasia was explored in relative depth. Fibrous dysplasia is a usually benign fibro-osseous abnormality of bone that may occur as monostotic, polystotic, or craniofacial disease or as a part of a syndrome. Its nosology is confusing and its etiology is still unknown.

Fibrous dysplasia commonly occurs in the military age group rendering these findings extremely important to the military medical practitioner.

#### NOONAN'S SYNDROME, REPORT OF A CASE WITH ORAL FINDINGS

A case of Noonan's syndrome in a 6-year old boy was studied in which zanthomas of the skin and tongue were an additional, not previously reported finding. The other syndromal stigmata are essentially those reported by

Noonan and others in the literature. This entity has only rarely been discussed in the dental literature although a great number have been reported in the medical literature.

#### DENTAL CHANGES IN FAMILIAL IDIOPATHIC HYPOPARATHYROIDISM

Three documented cases of diagnosed idiopathic hypoparathyroidism in siblings were studed. These cases are of interest due to their peculiar familial configuration and the paucity of all physically observable stigmata except those which involved the teeth.

The detention and treatment of cases such as these in a military medical center illustrates the multi-faceted function of military medicine.

#### SEM OF THE TOOTH-BONE INTERFACE USING CRYOMICROTOMY

order to establish structural standards for evaluation of satisfactory restructuring of healed oral surgical implants. Specimens of whole animals were quick-frozen in isopentane and sectioned with the LKB cyromicrotome. Thick-sections (20-40 µm) were freeze-dried, mounted, metal coated, and examined with light and scanning electron microscopy. Scanning microscopy revealed deep penetration of collagenous bundles into dental cementum, with orientation of apatite crystallites at right angles to the fiber bundles. This technique was found to be an important advance in rapid, precise specimen analysis of interfaces in calcified tissues allowing SEM study of calcified tissue without decalcification or freeze-fracturing.

This technique can be easily applied in research studies to the evaluation of satisfactory healing and reorganization of interfaces at

sites of surgical wound repair and surgical grafts or implants. The results of the SEM study of cemental attachment indicate that a successful repair of damage to cemental surfaces, or adequate functional attachment to oral surgical implants requires deep and calcified reinsertion of collagenous fiber bundles oriented perpendicular to the reattached surface.

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- 23. (U) To determine the efficacy and application of pressurized water lavage to the treatment and prevention of disease in traumatic wounds, oral maxillofacial surgery and military dentistry.
- 24. (U) Specially designed instruments which yield water jets at different pressures and in both pulsating and nonpulsating streams are being used to study the effect of this modality on wound healing, dental plaque, and bacterial populations. The target tissue pressures with various water jet devices will be examined. The applicability to presurgical hand cleansing is being investigated.
- 25. (U) (76 07-77 10) Continued study of bacterial contamination beneath fingernails following a conventional 10 minute scrub or 90 second hydroscrub lavage showed that significant bacterial residues remained in either case. Methods of eliminating this problem using hydroscrub are being studied. A study is in progress to improve the methodology for determining the effectiveness of iodine containing antiseptic agents used in hand degerming so that more accurate evaluations can be made of the role of these substances in surgical scrub. Attempts to use the free fatty acid levels on skin as a correlate of the effectiveness of surgical scrub or of the level of bacterial contamination of the skin, have not been successful. However the methods developed may be useful in the diagnosis of wound infections.

### ROLE OF PRESSURIZED WATER LAVAGE IN THE PRACTICE OF MILITARY DENTISTRY

### EFFECT OF SURGICAL PREPARATION OF HANDS ON BACTERIAL CONCENTRATIONS UNDERNEATH THE FINGERNAILS

Hospital acquired systemic and wound infections are significant problems. The reasons for spread of such infections are multiple, and there is no general agreement on what factors are the main hazards to the normal recovery of patients. Hand washing is recognized to be an important step in preventing the dissemination and transmission of potential pathogens. The US Army Institute of Dental Research has shown that the 10 minute conventional scrub can be replaced by a 90 second lavage using a new hand and arm washer (Hydroscrub) designed by the Institute with results as good or even better than those obtained by conventional methods. The extensive literature on surgical scrub completely ingnores the idea of determining the concentrations of microbial flora underneath the fingernails prior to and after surgical scrub. No data is available to answer this important question.

We have therefore initiated a study, to determine the efficacy of the two methods; 10 minute conventional scrub and 90 second Hydroscrub, in eliminating or reducing microbial numbers under the fingernails. The microbiological testing was done on members of the USAIDR staff. Determination of bacterial counts on 66 subungual areas before and after 10 minute conventional scrub has shown  $4.65 \times 10^4$  CFU (colony forming units) before and  $3.05 \times 10^4$  CFU/area after scrub. Counts on 64 subungual areas before and after 90 second Hydroscrub revealed  $2.36 \times 10^5$  CFU before and  $3.2 \times 10^4$  CFU/area after Hydroscrub.

Although the averages indicate there may be some reduction of microbial

population following pre-surgical hand preparation, the extremely high number of bacteria still remaining in subungual areas should alert every member of the surgical team to the possible danger of (until now) unrecognized, or ignored, failure of proper hand degerming prior to surgery. It should also be pointed out that in some subjects the cleansing procedure used significantly decreased the number of subungual bactera, while in others the count increased. This increase could be due to the exposure and disperson of the microflora from otherwise difficult to reach grooves and irregularities of the subungual areas. Lavage adjunctive methods of eliminating subungual bacteria are being pursued.

### COMPARISON OF IODINE INACTIVATING AGENTS IN SURGICAL SCRUB TESTING

In studies on the effectiveness of various antiseptic agents in hand degerming, different substances have been used for the purpose of neutralizing these agents. Such neutralization is often necessary prior to or during bacteriologic testing of the scrub method under investigation, because the carry-over of the antiseptic from hands onto the culture media may interfere with the growth of microorganisms, the numbers of which are an indication of the efficacy of a particular scrubbing method.

We have reported that the most commonly used inactivator of iodine, sodium thiosulfate, has a marked inhibitory effect on bacterial growth and concluded therefore, that results of bacteriological testing of iodine scrub preparations are misleading if sodium thiosulfate is incorporated in culture media. The implications of such false negative results obtained when testing the effectiveness of iodine scrub preparations in surgery and particularly in combat related surgical procedures, are obvious. This report presents incomplete results of testing the suitability of three iodine neutralizing agents used in studies on the effectiveness of iodine scrub preparations;

sodium thiosulfate, sodium sulfite, and D/E neutralizing medium. The antibacterial effect of these agents has been tested on six reference organisms, Streptococcus pyogenes, Streptococcus fecalis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, and on ten bacterial strains isolated from the skin of the fingertips of two subjects. Sodium thiosulfate and sodium sulfite were incorporated into the culture media in concentrations of 0.01 - 2.0 percent. D/E neutralizing medium was used as recommended by the manufacturer. The results have shown that the growth of reference organisms, S. pyogenes, S. fecalis, S. aureus, and S. epidermidis was inhibited by sodium thiosulfate. Sodium sulfite and D/E neutralizing medium did not inhibit the growth of these bacterial species. The two gram negative reference organisms, P. aeruginosa and E. coli were not inhibited by any of the agents.

Ten bacterial strains isolated from the fingertips of two subjects were affected to a different degree by the agents tested. All the strains, with two exceptions, were inhibited by sodium thiosulfate. Sodium sulfite appeared to be less inhibitory, but the growth of five strains, particularly at higher concentrations of sodium sulfite, was inhibited. Only two strains were inhibited when grown on D/E neutralizing medium.

No definite conclusions can be drawn at this time. Additional tests are necessary. It appears however that there is no "ideal" iodine neutralizing agent for the purpose of testing iodine scrub preparations. The results indicate however that D/E neutralizing medium compares favorably with the two other agents tested.

### THE DEVELOPMENT OF A RAPID HPLC METHOD FOR THE DETERMINATION OF FATTY ACIDS ON THE HANDS

It has been shown that certain fatty acids inhibit bacterial growth. This project was initiated to determine if people with low bacterial concentrations on their hands had unique fatty acids present on the skin of their fingertips. Our results showed poor correlation between free fatty acids (FFA) on the hands and bacterial counts. It was also found that following hand degerming with the use of pulse pressure water lavage, similar chromatograms could be consistently obtained and reproduced from individual to individual.

This finding may indicate that many FFA on the skin of healthy individuals are derived from the indigenous microflora, and that endogenous FFA are essentially the same although their levels on skin may vary slightly.

In one individual who has been shown to be a yeast carrier, chromatograms revealed unique fatty acids above C20 in chain length. This study has also shown that high pressure liquid chromatography is a very powerful tool in the studies of fatty acids on the skin and may be of great value in the study of dermatological disorders. The methodology used and the knowledge gained is being effectively applied to the investigations of early detection of wound infecting bacteria, which is of great significance to combat injuries and their treatment.

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- 23. (U) To develop simple, rapid methods of soft tissue or bone grafting of particular interest to the oral surgeon treating combat type maxillofacial injuries.
- 24. (U) The fate, metabolism, osteogenic potential and tissue compatibility of ceramic and copolymer materials will be studied alone and in combination. The application of these materials to avulsive type wounds in both animals and humans will be pursued.
- 25. (U) (76 07-77 10) Human studies on the osteogenic potential of degradable ceramic in alveolar bone defects are continuing. Results are very promising. A number of studies are in progress using dogs. An animal model (dog) has been developed to evaluate the osteogenic potential of graft materials suitable for alveolar bone reconstruction and a study is in progress comparing biodegradable ceramic against autogenous bone as alveolar ridge augmentation agents. Biodegradable ceramic is also being evaluated as a means of obliterating cystic cavities in facial bones disfigured by trauma. Preliminary results indicate the induction of osteogenesis. PIA is being evaluated as an osseous fixation device. All animals in the PIA and the ridge augmentation studies have been operated and repair will be followed for periods up to 24 weeks.

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### NEW AND IMPROVED TECHNIQUES FOR GRAFTS AND BONE REGENERATION

# OSTEOGENIC POTENTIAL OF DEGRADABLE CERAMICS IN ALVEOLAR BONE DEFECTS (HUMAN)

Presently USAIDR is conducting a research project in which biodegradable tricalcium phosphate is being utilized in osseous defects of humans. Previous reports have related the excellent results in dogs and monkeys, where the ceramic material was found to be very well accepted biologically and, when placed in bony defects, allowed new bone to form.

Ceramic implants have now been placed into a total of 32 different defects, and two defects which were apparent failures received additional implants. In accordance with the approved altered protocol requiring reentry procedures at 18 months, reentries were not accomplished during the period covered by this report.

Seventeen graft sites were reevaluated clinically and roentgenographically. Two sites showed complete bone fill; seven sites showed 50% or greater bone fill; and eight sites showed less than 50% bone fill. Neither root resorption nor ankylosis appeared to be occurring three and one-half years following implantation. During the period covered by this report, eight additional patients received implants in 13 sites. Reentry procedures will be performed in the prescribed 18 months. The ceramic implant material continues to hold great promise as a biodegradable implant material for alveolar bone regeneration.

# COMPARISON OF BIODEGRADABLE CERAMIC BLOCK AND AUTOGENOUS BONE GRAFTS AS ALVEOLAR RIDGE AUGMENTATION AGENTS WHEN INTERPOSED BETWEEN ALVEOLAR AND BASAL BONE

Because approximately ten percent of all combat injuries are maxillofacial in nature avulsion of alveolar and basal bone of the jaws is not
uncommon. A total of fifteen dogs have been operated, comparing the
results of ridge augmentation by interposing biodegradable ceramic blocks
or autogenous bone grafts between alveolar and basal bone in the mandible.
The animals will be sacrificed at 6, 12 and 24 weeks at which time a clinical
evaluation will be made of the net ridge augmentation obtained by this
technique. A histological study will employ a scaled assessment of osteogenesis present in each time period as well as the osteogenic configuration.
The first animal was scheduled to be sacrificed in September 1977.

#### EVALUATION OF A BIODEGRADABLE MESH FOR BONE GRAFTS IN THE DOG'S MANDIBLE

The reconstruction of facial bones following combat related avulsive wounds usually requires the use of nondegradable metal devices for intraosseous fixation. Six dogs were operated and a surgical defect of approximately two centimeters were created in the body of the right mandible.

A sheet of one hundred percent biodegradable polylactic acid was then
shaped and utilized as a tray to support a particulate and marrow bone
graft to the area. The tray was stabilized with four polylactic acid
screws which traversed the buccal aspect of the tray, the buccal and lingual
cortices of bone, and engaged the lingual aspect of the tray. All animals
are progressing satisfactorily. A four week specimen has been obtained,
however the histological evaluation has hot been completed. The four week

specimen revealed that the tray had separated from one screw and appeared to be deformed. However, the graft appeared to be healing satisfactorily. If successful, this type tray will offer the advantage of a biodegradable mesh to support grafts to facial bones and eliminate the necessity of a second operation for removal of a nondegradable appliance.

# OBLITERATION OF CYSTIC CAVITIES IN FACIAL BONES UTILIZING A BIODEGRADABLE CERAMIC MATERIAL

Asymmetrical facial bone contour is often a complication of maxillofacial trauma as seen in combat situations and/or tumor and disease management. Frontal bone and sinus involvement particularly, presents problems in
this regard in military missile wounds. Obliteration of the sinus cavity
itself or bone fragment avulsion with autogenous grafts or biodegradable
osteogenic agents presents as a possibility if frontal bone contour can be
controlled while the osteogenesis progresses.

The purpose of this study is to induce an osseous filling or obliteration of the frontal sinus in dogs while providing an acceptable esthetic contour over the experimentally obliterated cavity wall.

Preliminary findings in this study indicate that powdered biodegradable ceramic induces osteogenesis although not all data has not yet been compiled.

### THE DEVELOPMENT AND EVALUATION OF THE ALVEOLAR BONE GRAFT TECHNIQUE

An animal model system was developed to evaluate the osteogenic potential of graft materials suitable for alveolar bone reconstruction subsequent to combat injuries. The mandibular right and left second and fourth premolar teeth were extracted on each of six dogs. The extraction sites were

modified to a standard design. Reconstruction techniques included:

- a. a sheet of polylactic acid (PLA) fitted over the defect to conform to the preexisting ridge height.
  - b. packing the defect with marrow then using PIA as in "a" above.
  - c. packing the defect with marrow.
  - d. no further treatment (controls)

The gingiva was approximated and closed over all defects. Postoperative complications included breakdown of the suture line over the PIA sheet however, the PIA was retained and subsequently covered over by epithelium. The sacrifice period was three months. At sacrifice the mandibles were disected free and the soft tissues removed. Direct measurements were taken to compare the presurgical alveolar bone height to the 3 month postoperative height, a slight increase in crestal bone height was noted in the groups with PIA. The mucosal breakdown over the PIA implants was judged to be due to the large size of the sheet (approximately 2x2 cm) resulting in ischemia. The tissue compatability of the PIA was excellent. This is a final report.

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- 23. (U) Army endodontic procedures in the military total 108,000 per year and are 25% of dental emergency procedures. Tooth reimplantation with endodontic therapy is involved in most serious facial injuries and involved typically 3 to 5 patient visits. The military can gain at least 50% reduction in patient and specialist man-hours spent in endodontic therpay with the development of more rapid and reliable treatment materials and techniques.
- 24. (U) Two areas to be investigated under this project are: (1) analysis of endodontic materials including those in use and newly developed; (2) techniques used in endodontic therapy with emphasis on the development of the most rapid and accurate method within the military type practice.
- 25. (U) (76 07-77 10) The toxic potential of the minor elements in amalgam used in an endodontic context is being studied by SEM and microprobe analysis in rats and in human oral biopsy materials. The data suggest that tin and sulfur may be involved in long term pathologic responses. Some critical factors in the effectiveness of the endodontic sealing technique have been determined by SEM analysis. Retention of endodontically treated roots as a means of preserving the alveolar process appears to be a viable procedure. An electrically heated instrument holder developed by USAIDR is undergoing clinical evaluation. The objective is to supply more predictable temperatures in endodontic procedures requiring heated instruments.

### DEVELOPMENT OF ENDODONTIC PROCEDURES FOR MILITARY DENTISTRY

#### SEM AND MICROPROBE ANALYSIS OF BONE RESPONSE TO ZINC-AMALGAM IMPLANTS

Freshly mixed, unset zinc-free and zinc-containing amalgam was implanted in the right tibia of 32 rats. Half of the specimens were examined by the light microscope and the other half by the scanning electron microscope and x-ray microprobe analysis. It was found that there was no histological reaction differences between zinc and zinc-free amalgam.

The surfaces of the implants were covered by an organic film at three weeks, and with bone at later intervals. Very little corrosion products containing sulfur were observed on the amalgam surface at all intervals. Bone adjacent to the amalgam contained tin and sulfur irrespective of the presence of zinc in the alloy, indicating outward migration of specific components of the alloy.

In military dentistry, because of the great specialization of dental care delivery, it is of prime importance to evaluate potential hazards to certain of these specialists, namely those concerned with placement of dental amalgam restorations. Mercury toxicity has been extensively studied; however, other components of the amalgam, silver, zinc, tin, and copper, require more investigation. In addition, data from this study will be applied to evaluation of hazards resulting from metal fragments in combat wounds, lodged in maxillofacial tissues.

AMALGAM PARTICLES IN HUMAN ORAL TISSUE - SEM AND X-RAY MICROPROBE STUDY OF METAL MIGRATION INTO ADJACENT TISSUE (1)

Migration of tin, sulfur, and zinc into bone adjacent to amalgam implants, as a result of a previous experiment indicated that similar results would be observed in cases of amalgam "tattoo" or implantation into oral tissue of dental amalgam. This investigation was considered important in that amalgam is used in the military patient as a retrograde endodontic sealant material, is therefore available for incorporation into oral tissues during surgery and is already available as study material in USAIDR biopsy material. Therefore, oral biopsy material containing amalgam was analyzed in the SEM-x-Ray system for relative elemental concentrations in various areas within and adjacent to amalgam tissue "implants". Tin and sulfur were found to be selectively concentrated at the interface between amalgam and tissue. These results indicate that tissue response over long term reactions are concerned with biochemical reaction to tin and sulfur, and that mercury and silver, principal components of the amalgam are of less importance in any subsequent pathologic response.

This report is one of a series of a study of potentially toxic hazards to the military dentist and patient population resulting from dental procedures and materials. Data from this study will be utilized in evaluating tissue response to combat surgical wounds involving incorporation of metal fragments in the wound site.

#### SEALING QUALITY OF A TEMPORARY FILLING MATERIAL

The function of a temporary filling material in military endodontics is twofold: first, to prevent the saliva with is microorganisms from gaining entrance into the root canal, thus preventing infection or reinfection; second, to prevent medicaments placed in the pulp chamber from escaping into the oral cavity, thereby preserving the effectiveness of the intracanal medication and preventing any chemical burn to the oral mucosa.

If these criteria are to be met, the sealing qualities of a temporary filling material are of primary importance in endodontic therapy.

Patients undergoing endodontic treatment at military facilities occasionally complain of a foul taste or a burning sensation. This is evidence of an inadequate seal even though leakage is not apparent on visual examination. This study was designed to evaluate the thickness of Cavit necessary to achieve an appropriate seal.

Endodontic access cavities were prepared in forty extracted human teeth. The access cavities were obturated with Cavit and tested for leakage with Methylene blue. The data suggested that at least a 3.5 mm thickness of Cavit should be used in order to prevent leakage. Examination under the scanning electron microscope showed areas in which the constituents of Cavit were improperly mixed. This may lead to increased penetration. There were 189,000 endodontic procedures done by Army dentists in 1976. Any substantial improvement in technique will save dollars and time.

### THE EFFECTS OF OVERFILLED POLYETHYLENE TUBE INTRAOSSEOUS IMPLANTS ON RAT OSSEOUS TISSUE

In endodontic treatment the obliterating materials may terminate short of the apex, flush with it, or extend into the periapical tissues. In the latter instance these are usually referred to as overfillings. Since these materials remain permanently embedded, a knowledge of the biocompatibility of the materials used in obliterating root canals is essential for successful endodontic treatment.

Forty-eight white male Walter Reed rats were used to study the osseous tissue inflammatory response to polyethylene tubing filled 2mm long on one end and flush at the other end with unset Grossman's cement and gutta percha.

One of these tubes was placed in one tibia while the other tibia received a hollow polyethylene tube as a control. The animals were sacrificed in six groups of eight each at 4, 7, 14, 30, 60 and 90 days following tube insertion. Histologic examination of the specimens revealed the following:

- The gutta percha, Grossman's sealer and polyethylene tubing were well tolerated by rat intraosseous tissue.
- 2. A statistically significant difference in the amount of inflammation was noted in the 7-day experimental group as compared with the controls. This difference was thought to be the result of the irritating properties of unset Grossman's sealer.
- The overfillings per se did not significantly compromise the healing of rat intraosseous tissue.

These findings of irritation by a commonly used root canal sealer and that overfilling does not affect results will save professional and patient time and therefore dollars.

#### RETENTION OF ROOTS TO PRESERVE THE ALVEOLAR PROCESS

One year following retention of roots procedure on one patient, the procedure appears to be 100% successful. Radiographically, there are no areas of pathology or alveolar bone loss. Clinically, all tissues appear to be healthy and the patient has had no symptomatology. There continues to be a scarcity of patients due to the selectivity required and although requests have been made to other dental services at Ft. Meade we are still seeking additional patients to undergo this procedure.

AN ELECTRICALLY HEATED INSTRUMENT HOLDER FOR USE IN TOOTH BLEACHING AND ENDODONTICS AND FOR GENERAL UTILITY IN FIELD AND CLINICAL DENTISTRY

Many procedures in dentistry must be, or are best done with heated instruments. The usual procedure is to carefully heat an instrument over an open flame and trust that it will be at the proper temperature when it reaches the operating site. This is a cumbersome, inexact procedure, often involving the mutilation of quality instruments which must then be replaced. Development of a convenient, electrically heated holder capable of accommodating a wide assortment of operating tips would enable the dental officer to function precisely, efficiently and economically, with no need to damage instruments needed elsewhere. This is especially advantageous in field practice, where circumstances and minimal available time and material demand economy in both function and instrumentation.

As previously reported a prototype instrument was designed and constructed. During the past year the design has undergone several changes and 5 additional instruments were constructed and are now in clinical evaluation. Further refinements of the design based on preliminary clinical evaluations are now in progress.

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Deemer, J.P, Tsaknis, P.J., Ayer, W.A.: The Effects of Overfilled Polyethylene Tube Intraosseous Implants on Rat Osseous Tissue. Oral Surg. In Press.

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- 23. (U) To determine the best usage of biodegradable copolymer materials for the treatment of both hard and soft tissue wounds. To develop premedicated biodegradable tissue fixation devices. To determine feasibility of a long-term, subcutaneous, slow release drug delivery system.
- 24. (U) The recent adaptation by USAIDR of the biodegradable copolymers to the surgical procedures such as tendon gliding devices, oral antral fistula closure and peripheral nerve repair will be expanded in animals and extended to man. Application to the Drug Review Board has been made for this usage.
- 25. (U) 976 07-77 10) A collagen artificial skin used as an oral wound covering was found to be an effective treatment modality. Biodegradable copolymers of PLA/PGA and biodegradable ceramic used singly and in combination in experimental bone wounds in rats indicated that ceramic provided a somewhat faster and more uniform osteogenesis than the copolymers. Segmental urethral grafts in dogs from preformed autogenous connective tissue cylinders formed around degradable copolymer cuffs have been partially successful. Segmental urethral grafts using copolymer tubes have progressed satisfactorily in 3 of 4 dogs. Observations continue. Segmental esophageal grafts with copolymer tubes in rats have not been successful. A flexible burn dressing made of copolymer is being evaluated in rats. A study on the carcinogenic potential of biodegradable ceramic has thus far (9 months) been negative.

vellable to contractors upon originator's aggreval.

#### BIODEGRADABLE MATERIALS FOR USE IN TREATMENT OF FRACTURES AND WOUNDS

### WOUND HEALING OF THE ORAL SOFT TISSUES USING COLLAGEN ARTIFICIAL SKIN

The purpose of this study was to determine the biocompatability of collagen artificial skin in the oral mucosa. The material used in this investigation is an enzyme-solubilized calfskin which is individually prepared in sheet form.

Sixteen new Zealand white rabbits of mixed sex, weighing 4-5 lbs. were used. A recipient site was prepared by scalpel excision in the mucobuccal vestibule on the maxillae of each animal. A section approximately 2x2 cm of epithelium, connective tissue and underlying subcutaneous fat was removed while the periosteum was left intact. A piece of collagen artificial skin was cut to size and sutured over the prepared wounds. An identical wound was prepared contralaterally. However, no biomaterial was placed. This served as a control.

Following sacrifice, tissue preparation and microscopic evaluation observations were made which determined that the five day specimens revealed surgical wounds covered by an opaque coagulum of soft tissue. Attempts at removing the granulation plug indicated that the wound was well organized and firmly established to the underlying and surrounding mucosa. The mucosal periphery assumed a normal color and there was no evidence of edema in the area.

Succeeding time periods of 10, 14, and 21 days demonstrated a subsequent reduction of inflammatory cells and of the vascularity. A continuous contraction of the wound occurred with formation of mature fibrous connective tissue, eventual re-epithelialization and complete unremarkable healing. Throughout the various sacrifice periods, no significant clinical or microscopic difference was observed between the experimental and the control specimens.

It is concluded that the collagen artificial skin does not produce undesirable effects in the healing wound and may therefore be a valuable adjunct in oral wound healing with respect to patient comfort and the prevention of trauma to susceptible areas during food intake and thus facilitate adequate nutrition.

### EVALUATION AND COMPARISONS OF BIODEGRADABLE SUBSTANCES AS OSTEOGENIC AGENTS

A solution to the problem of sinus and bony resolution and esthetic disfigurement following combat-induced trauma are important considerations in military research.

This study compared the osteogenic potential and tissue compatibility of biodegradable copolymers-PLA/PGA and a biodegradable ceramic-Ca<sub>3</sub> (PO<sub>4</sub>)<sub>2</sub>. These compounds were placed in experimentally created defects in rat tibias, both in combination and singly, and evaluated at 14, 28, and 42 days. The ceramic served as a format to result in uniform osteogenesis throughout the defect. The copolymer implants resulted in a more gradual bone formation, progressing slowly from the wound peripheries. The ceramic and copolymer combination behaved little differently from the copolymer alone. All experimental materials were extremely tissue tolerant, with minimal inflammation and no foreign-body reactions.

### SEGMENTAL URETERAL GRAFTS (NEOGENESIS)

Six dogs were operated and a left ureteral segment was replaced utilizing a preformed connective tissue hollow cylinder. The tissue cylinders were formed subcutaneously in the abdominal wall by allowing degradation of a copolymer cuff and consequent replacement by fibrous connective tissue. These tissue cylinders were initially successful in the 6 dogs but failed between 3 and 6 weeks due to dystrophic calcification of the transplanted autogenous tissue cylinder.

Another attempt will be made to isolate a blood vessel and carry the vessel to the recipient site and anastomose it with a nearby blood vessel.

This partially successful technique can be applied to any hollow organ and improvement of the technique will continue.

#### SEGMENTAL URETERAL GRAFTS

Four dogs have received segmental ureteral replacements with a preformed "intertwined" copolymer hollow cylinder.

Three of the four animals are presently progressing satisfactorily and in 2 who have had post recovery I.V.P.'s show functioning kidneys and filling of the ureter to the bladder. The 4th animal died at 3 weeks of a combined infection and ureteral constriction.

This project is still in progress.

Future success of this technique and material would allow direct replacement of hollow organs.

#### SEGMENTAL ESOPHAGEAL GRAFTS

A pilot study replacing 8 rat esophagi with preformed "intertwined" copolymer hollow cylinders has shown early success in all animals but a gradual blockage at approximately 3 weeks. One animal did well until 24 days.

Histological studies show a new lining of epithelial cells and blockage by foreign material such as hair, food and mucous.

Numerous techniques and materials were given trials in attempts to keep the graft open.

The project will be continued in a larger animal to attempt to avoid blockage by food, etc.

#### BURN AND WOUND DRESSING

A semipermeable, dense, flexible burn dressing has been prepared from a copolymer and placed on ten rat backs in an attempt to determine its ability to adhere to tissue, allow transpiration, resist infectious organisms and encourage connective tissue ingrowth.

Twenty samples from the 10 rats are presently being prepared for histopathology review.

Clinical results appear encouraging and the study will be continued.

#### CARCINOGENESIS STUDY ON BIODEGRADABLE CERAMIC

The F.D.A. requested a 2 year study on rats of the degradable ceramics material presently being used in USAIDR's human study.

The study is at the 9 months duration and progressing well.

No clinical evidence of any deleterious health effect has been observed.

Daily observations and notebook entries are being kept.

#### **PUBLICATIONS**

Levin, M.P., Tsaknis, P.J.: Wound Healing of the Oral Soft Tissues Using Collagen Artificial Skin. J. Dent. Res. 56: (Special Issue B) 1977.

Miller, R.A., Brady, J.M. & Cutright, D.E.: Degradation Rates of Oral Resorbable Implants (Polylactates and Polyglycolates): Rate Modification with Changes in PLA/PGA Copolymer Ratios. J. Biome. Mater. Res. 11: 711-719 1977.

Reid, R.L., Cutright, D.E. and Garrison, J.S.: Biodegradable Cuff an Adjunct to Peripheral Nerve Repair: A Study in Dogs, <u>The Hand</u>, (Submitted 14 Sep 77).

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